9
Biotic Influences

9A. Symbiotic Associations

1. Introduction

Symbiosis is the "living together" of two or more organisms. In its broadest sense, symbiotic associations include parasitic and commensal as well as mutually beneficial partnerships. As is common in the ecophysiological literature, however, we use the term **symbiosis** in a narrow sense to refer to **mutually beneficial associations** between higher plants and microorganisms. Mutual benefits may not always be easy to determine, particularly for the microsymbiont. In this chapter benefits for the macrosymbiont ("host") are often expressed in terms of ability to accumulate biomass. In an ecological context, benefits in terms of "fitness" may be more relevant but are rarely documented. In the mutually beneficial associations discussed in this chapter, nutrients or specific products of the partners are shared between two or three partners; the macrosymbiont and the microsymbiont(s). Parasitic associations between higher plants are dealt with in Chapter 9D; parasitic associations between microorganisms and higher plants are discussed briefly in this chapter, and more elaborately in Chapter 9C on effects of microbial pathogens.

In Chapter 6 on mineral nutrition, we discussed numerous special mechanisms that allow some higher plants to acquire sparingly soluble nutrients from soils (e.g., excretion of carboxylates and phytosiderophores). We also pointed out (Sects. 2.2.5 and 2.2.6 of Chapter 6 on mineral nutrition) that some species are quite capable of growing on soils where P is sparingly available, without having a large capacity to excrete protons or carboxylates. How do these plants manage to grow? It is also obvious that special mechanisms to take up nutrients (e.g., N) are of little use, if the N is simply not there. Such plants must have alternative ways to acquire N.

This chapter discusses associations between higher plants and microorganisms that are of vital importance for the acquisition of nutrients. Such symbiotic associations play a major role in environments where the supply of P, N, or immobile cations limits plant growth. In the **rhizosphere** (or elsewhere in the plant's immediate surroundings), mycorrhiza-forming fungi and N_2-fixing bacteria or cyanobacteria may form symbiotic associations. For those species that are capable of such symbioses, it tends to be profitable for both the higher plant (**macrosymbiont**) and the microorganism (**microsymbiont**). Indeed, it is so profitable for the macrosymbiont that some plants are associated with more than one microsymbiont at the same time.

2. Mycorrhizas

Vast majority of higher plant species can form symbiotic associations with **mycorrhizal fungi**. Mycorrhizas are the structures arising from the association of roots and fungi; except for nonmycorrhizal species (Sect. 2.2), roots in soil should be considered in conjunction with their mycorrhizal symbionts. There are four main types of mycorrhizas (Sect. 2.1; Smith &

H. Lambers et al., *Plant Physiological Ecology*, Second edition, DOI: 10.1007/978-0-387-78341-3_9,

Table 1. The length of mycorrhizal hyphae per unit colonized root length as measured for a number of plant species, infected with different arbuscular mycorrhiza-forming fungal species.

Fungus	Host	Hyphal length (m cm^{-1} root)
Glomus mosseae	*Allium cepa (onion)*	0.79–2.5
Glomus mosseae	*Allium cepa*	0.71
Glomus macrocarpum	*Allium cepa*	0.71
Glomus microcarpum	*Allium cepa*	0.71
Glomus sp.	*Trifolium sp. (clover)*	1.29
Glomus sp.	*Lolium sp. (ryegrass)*	1.36
Glomus fasciculatum	*Trifolium sp.*	2.50
Glomus tenue	*Trifolium sp.*	14.20
Gigaspora calospora	*Allium cepa*	0.71
Gigaspora calospora	*Trifolium sp.*	12.30
Acaulospora laevis	*Trifolium sp.*	10.55

***Source*: Various authors, as cited in Smith & Gianinazzi-Pearson (1988).**

Read 2008). The most ancient type dates back to the early Devonian, some 400 million years ago (Nicholson 1975, Cairney 2000). The first bryophyte-like land plants had endophytic associations resembling **arbuscular mycorrhizas** (**AM**), even before roots evolved. The **ectomycorrhizal** symbiosis has evolved repeatedly over the last 130–180 million years (Martin et al. 2001). Like root hairs (Sects. 2.2.1 and 2.2.5 of Chapter 6 on mineral nutrition), the mycorrhizal associations enhance the symbiotic plant's below-ground absorbing surface. For some mycorrhizas (Table 1), this is the primary mechanism by which mycorrhizal plants are able to acquire scarcely available, poorly mobile nutrients, especially P (Sect. 2.1). For other mycorrhizas, additional mechanisms, such as excretion of hydrolytic enzymes and carboxylates, may also play a role (Sect. 2.1).

The mycorrhizal associations generally enhance plant growth, especially when P or other immobile nutrients limit plant growth; they may also be beneficial when water is in short supply and suppress infection by parasitic plants (Sect. 2.1 of Chapter 9D on parasitic associations). As such, mycorrhizas are of great ecological and agronomic significance. When the nutrient supply is high, however, they are a potential carbon drain on the plant, providing less benefit in return. Plants, however, may have mechanisms to suppress the symbiotic association at a high supply of P (Sect. 2.3.1).

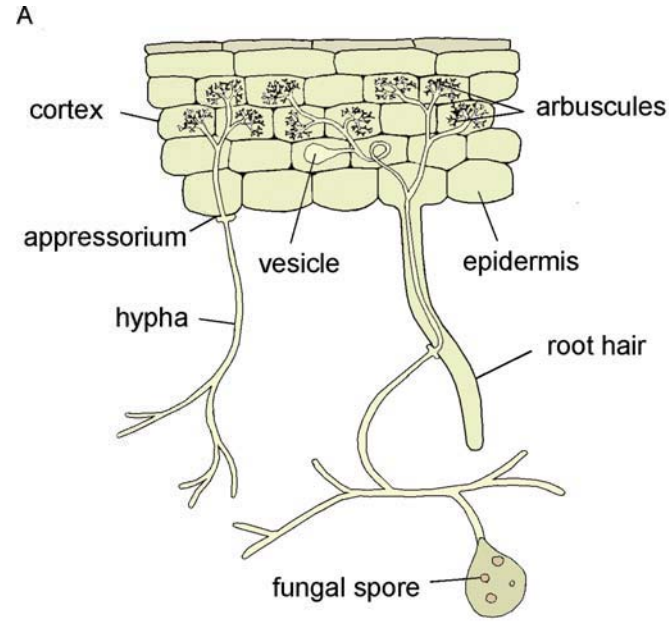
Some species never form a mycorrhizal association, even when P is in short supply. Some of these (e.g., Proteaceae; Sect. 2.2.5.2 of Chapter 6 on mineral nutrition) perform well when P is severely limiting; other nonmycorrhizal plants may even be harmed by mycorrhizal fungi (Sect. 7 of Chapter 9E). On the other hand, some nonmycorrhizal plants severely inhibit the growth of mycorrhizal hyphae. Before dealing with these complex interactions (Sect. 2.2), we discuss some general aspects of mycorrhizal associations.

2.1 Mycorrhizal Structures: Are They Beneficial for Plant Growth?

Mycorrhizas occur in 82% of all angiosperm species investigated to date; all gymnosperms are mycorrhizal (Brundrett 2002). A mycorrhizal association consists of three vital parts:

1. The root
2. The fungal structures in close association with the root
3. The external mycelium growing in the soil

Figure 1. (A) Schematic structure of an arbuscular mycorrhiza (AM). (B) Arbuscules (A) and a vesicle (V) of *Glomus* sp. colonized on the root of *Tagetes patula* (marigold). (C) Arbuscules (A) and intercellular hyphae (IH) of *Glomus etunicatum* isolated from the root of *Tagetes patula* after enzymatic digestion. (D) Detail of the intraradical hyphae of *Glomus mosseae* in a root of *Tagetes patula* (marigold), after enzymatic digestion of the root, showing fine branches and trunks of an arbuscule (Ezawa et al. 1995) (courtesy T. Ezawa, Faculty of Horticulture, Nagoya University, Japan).


A
cortex
arbuscules
appressorium
vesicle
epidermis
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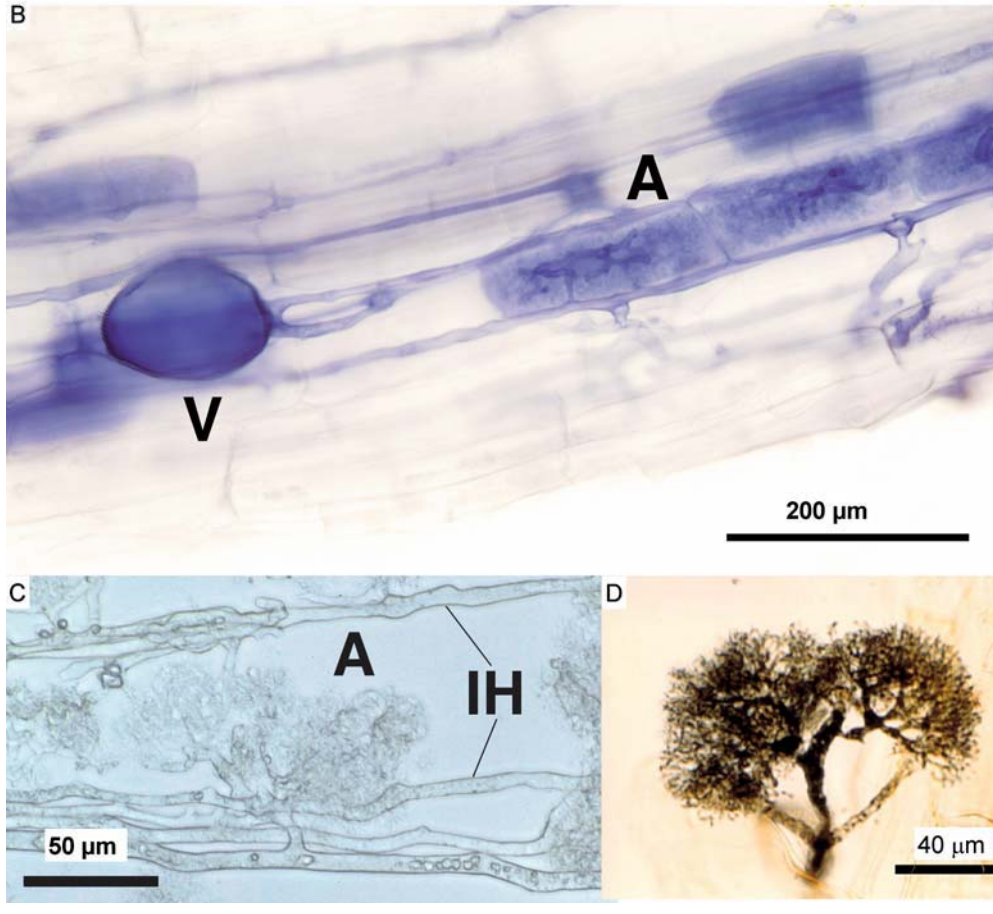
root hair
fungal spore


B
A
V
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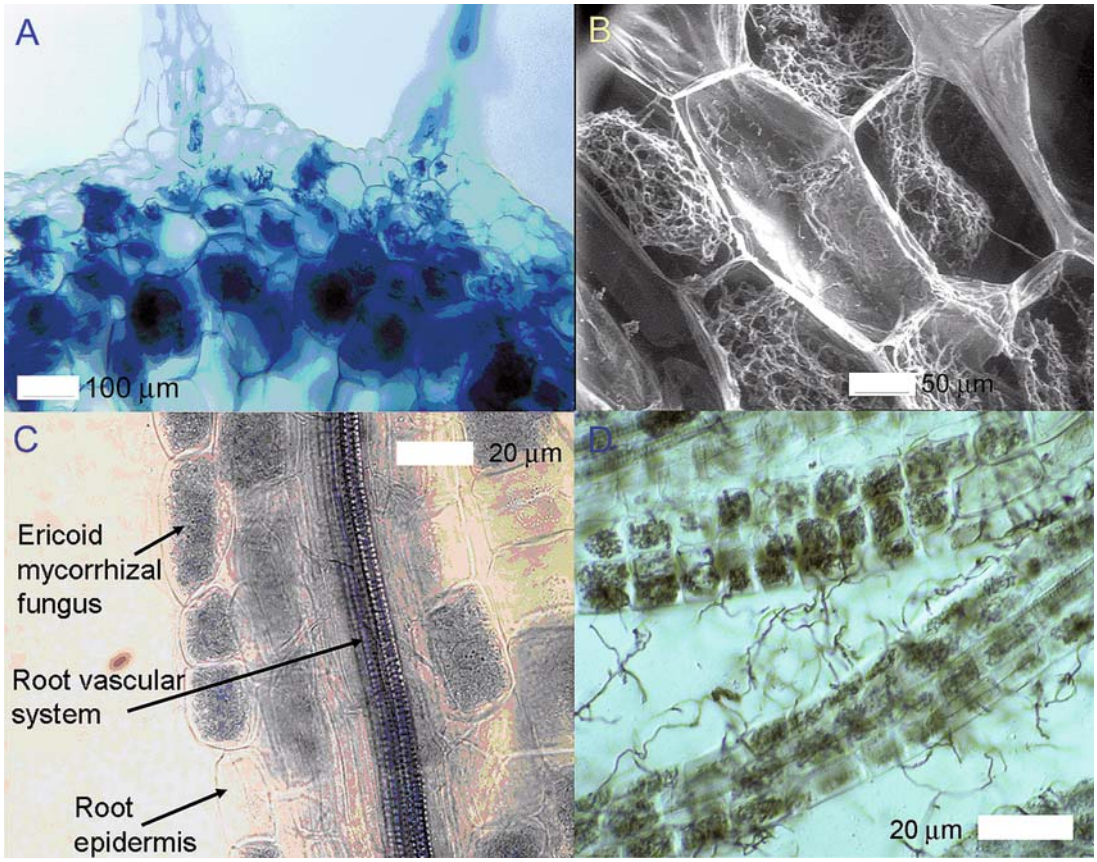
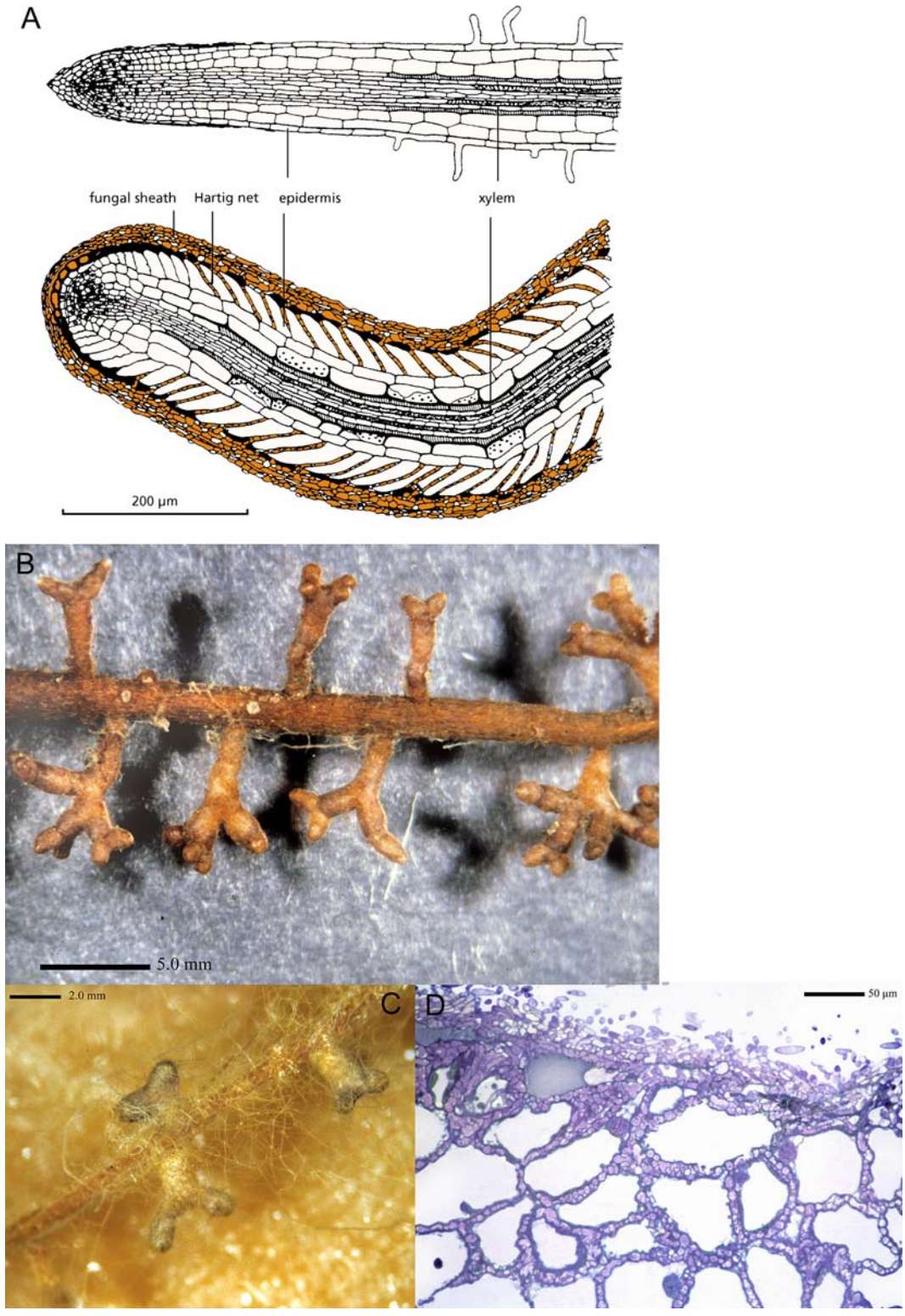


FIGURE 2. (A) Orchidaceous mycorrhizal association in a stem of *Pterostylis sanguinea* (greenhood orchid). (B) Transverse section of a stem of *Caladenia arenicola* (carousel spider orchid) showing intracellular fungal coils (courtesy A.L. Batty and M.C. Brundrett, The University of Western Australia, Australia). (C) Ericoid mycorrhizal association of *Woollsia pungens*, showing epidermal cells colonized by coils of an ericoid mycorrhizal fungus (stained blue, arrowed) (courtesy S. Chambers and J.W.G. Cairney, Centre for Plant and Food Science, University of Western Sydney, Australia; copyright Elsevier Science, Ltd.). Ericoid mycorrhizal association of *Leucopogon verticillatus* (courtesy M.C. Brundrett, The University of Western Australia, Australia).

Mycorrhizas are classified in different types, but some are very similar (Brundrett 2002). **Arbuscular mycorrhizas** (**AMs**) are the most widespread. A large fraction of the fungal tissue is within root cortical cells, outside their plasma membrane (Fig. 1). They frequently occur on herbaceous plants, but are also found on trees, especially in tropical forests. **Ericoid mycorrhizas** in the Ericaceae and **orchid mycorrhizas** in the Orchidaceae have somewhat different structures and functions (Fig. 2). *Alnus* (alder), *Cupressus* (cypress), *Eucalyptus* (eucalypt), *Fraxinus* (ash), *Populus* (poplar), and *Salix* (willow) are genera that have both AMs and **ectomycorrhizas** (**ECMs**) (Fig. 3; Brundrett 2002).

FIGURE 3. (A) Schematic representation of an ectomycorrhiza, showing the fungal mantle around the root and the hyphae in the cortex, which form the Hartig net. (B–D) Ectomycorrhizal association between *Pinus resinosa* (red pine) and an unknown fungal species. A higher magnification of *Pinus resinosa* and *Pisolithus tinctorius* as the mycobiont, showing thickened branched rootlets, covered in a fungal mantle, and external hyphae. The highest magnification is of a longitudinal section of a *Pinus resinosa–Pisolithus tinctorius* mycorrhizal root showing mantle hyphae on the root surface and Hartig net hyphae surrounding epidermal and cortical cells (courtesy R.L. Peterson, University of Guelph, Canada).


A
fungal sheath
Hartig net
epidermis
xylem
200 µm
B
5.0 mm
2.0 mm
C
D
50 µm

Less than 200 fungal species form AM; they are the most widespread mycorrhizal association (Nicholson 1975, Brundrett 2002). AMs are classified in six genera within the Glomeromycota, with *Glomus* being the largest genus. These fungi are considered to be "primitive" because they have relatively simple spores and they associate with a wide range of plant species. They are not capable of growing without a plant host. The AMs have been named after the **arbuscules** (which are tree-like structures that occur inside root cortical cells; Fig. 1). Although the arbuscule can fill most of the cell space, it does not compromise the integrity of the plant plasma membrane, because cortical cells envelop the arbuscule in a specialized host membrane, the **periarbuscular membrane** (Javot et al. 2007). The roots of 80% of all surveyed plant species and 92% of all families can be infected with AM-forming fungi (Wang & Qiu 2006). Even species that are typically ectomycorrhizal form AM associations in the absence of ectomycorrhizal inoculum.

In ericoid and orchid mycorrhizas, like in AMs, a large fraction of the fungal tissues is within the root cortical cells. In **ectomycorrhizas**, however, most fungal tissue is outside the root. This symbiotic association is frequently found between trees (Dipterocarpaceae: 98%; Pinaceae: 95%; Fagaceae: 94%; Myrtaceae: 90%; Salicaceae: 83%; Betulaceae: 70%; Fabaceae: 16%) and more than 5000 species that belong to the Basidiomycota (agarics, bolets) or Ascomycota (truffles) (Barker et al. 1998, Martin et al. 2001). Fossil ectomycorrhizas have been found among plant remains of *Pinus* (pine) from the Middle Eocene 40 million years ago (LePage et al. 1997). Although ectomycorrhizas occur mostly in woody angiosperms and Pinaceae, they have also been found in some monocotyledons and in ferns. **Ectomycorrhizas** independently evolved many times through **parallel evolution**. Co-evolution between plant and fungal partners in ECM has probably contributed to diversification of both plant hosts and fungal symbionts (Wang & Qiu 2006).

2.1.1 The Infection Process

Root exudates from AM host plants enhance, but are not required for spore germination, whereas exudates from nonhost plants, e.g., *Lupinus* (lupin) or *Brassica* (cabbage) species, do not stimulate germination. Roots of host plants also release a signal or signals that stimulate(s) the directional growth of the AM fungus toward them. CO_2 may be one such signal (Bécard et al. 2004), but there are others, analogous to the signal molecules (flavonoids) involved in the legume–rhizobium recognition interactions (Sect. 3.3; Scervino et al. 2005, Catford et al. 2006). In fact, flavonoids have been implicated in the recognition between AM hosts and fungi (Harrison 2005, Hause & Fester 2005). However, root exudates of *Zea mays* (corn) mutants deficient in chalcone synthase, which is an enzyme involved in flavonoid synthesis, show similar AM colonization as those of the wild-type (Bécard et al. 1995). This suggests that flavonoids are not the key components in the AM fungus–host regonition (Buee et al. 2000, Harrison 2005). In one of the first stages of AM **host recognition**, the hyphae of AM fungi show extensive branching in the vicinity of host roots in response to signaling molecules (Fig. 4). Root exudates contain a **branching factor** identified as a **strigolactone**, 5-deoxy-strigol (Akiyama et al. 2005, Paszkowski 2006). Strigolactones are a group of sesquiterpene lactones, previously isolated as seed-germination stimulants for the **parasitic weeds** *Striga* and *Orobanche* (Sect. 2.1 of Chapter 9D on parasitic associations). Strigolactones induce extensive **hyphal branching** in germinating spores of the AM fungus *Gigaspora margarita* at very low concentrations (Bouwmeester et al. 2007). They also play a role in monocotyledonous species [e.g., *Sorghum bicolor* (millet)], interacting with AM fungi at concentrations as low as 10^{-13} M. Within 1 hour of exposure, the density of mitochondria in the fungal cells increases and their shape and movement changes dramatically which is associated with a rapid increase of mitochondrial density and respiration (Besserer et al. 2006). Isolation and identification of plant symbiotic signals open up new ways for studying the molecular basis of plant–AM fungus interactions. This discovery also provides a clear answer to a long-standing question on the evolutionary origin of the release from host roots of molecules that stimulate seed germination in parasitic plants (Sect. 2.1 of Chapter 9D on parasitic associations; Akiyama & Hayashi 2006). Similar signaling between host and fungus also plays a role in mycorrhizal associations other than AM, but we know much less about this (Martin et al. 2001).

During the establishment of AM, fungal hyphae that grow from spores in the soil or from adjacent plant roots contact the root surface, where they differentiate to form an **appressorium** in response to signals released from the host roots, and initiate the internal colonization phase (Genre & Bonfante 2005). Appressoria form only on epidermal cells, and generally not on the roots of nonhost plants, a further indication of recognition signals (Harrison 2005). Penetration of the root occurs via the appressoria, and the fungus frequently enters by forcing its

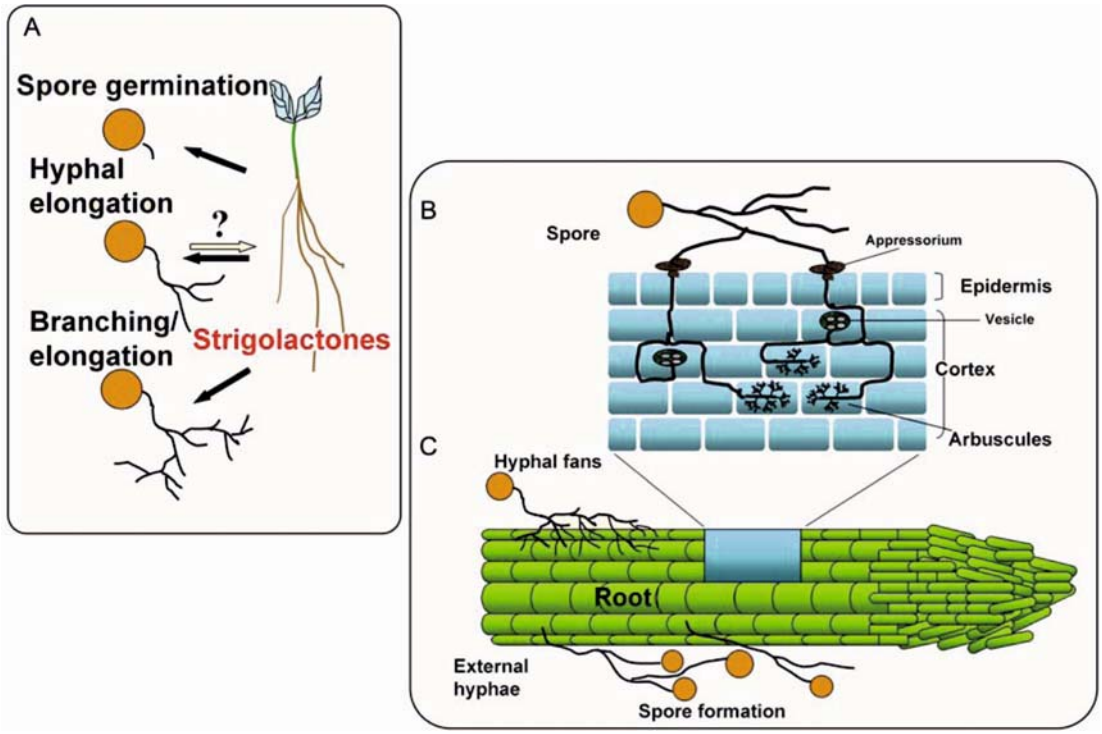


FIGURE 4. The complete life cycle of arbuscular mycorrhizal fungi, involving recognition, communication, and establishment of symbiosis between fungus and host. The pre-germination stages may be stimulated by the plant root exudates, but may also occur in its absence (after Gadkar et al. 2001).

way between two epidermal cells. Alternatively, a hypha may penetrate the cell wall of an epidermal or root-hair cell and grow through the cell as a result of localized production of hydrolytic enzymes by the fungus.

Once inside the root, the fungus produces intercellular hyphae, coils, and **arbuscules**. The function of the arbuscules is most likely to increase the surface area of membranes over which exchange of metabolites occurs and so to enhance active transport between the plasma membrane of the host and the hyphae of the fungus. The invaginated membranes are highly specialized; they contain mycorrhiza-inducible **P_i transporters** (Rausch et al. 2001, Karandashov & Bucher 2005) and **H^+-pumping ATPases** (Ferrol et al. 2002, Requena et al. 2003). Arbuscules are short lived and usually degenerate within a week or two. Thus, progression of colonization requires continuous arbuscule formation as the fungus spreads in the roots (Gadkar et al. 2001). The hyphae proliferate both in the cortex and in the soil. **Vesicles**, in which lipids are stored, are sometimes formed at a later stage, either between or within cells. The AM fungus does not penetrate into the endodermis, stele, or meristems; the fungus usually colonizes roots where the endodermis does not have a complete suberin barrier yet (Brundrett 2002).

The structure of orchid mycorrhizas have also been studied intensively; as with AM, there is extensive intracellular growth with fungi forming intracellular **fungal coils**, rather than arbuscules (Fig. 2). The fungi forming the mycorrhizas are Basidiomycota and many belong to the genus *Rhizoctonia*. As soon as they have germinated, the orchid seedlings, which have very few reserves, depend on organic matter in the soil or from other host plants which is supplied via the mycorrhizal fungus. *Rhizoctonia* species may form associations with both orchids and conifers. The orchids are therefore not saprophytic, but **mycoheterotrophic** (i.e., parasitic on the fungus) (Leake 2004); the association between host and fungus does not appear to be mutually beneficial. Even orchids that have the ability to photosynthesize may form ECM with forest trees, and their stable N- and C-isotope signatures indicate a dependence on ECM. This would explain the success of orchids in low-light environments (Bidartondo et al. 2004). In those orchids that remain nonphotosynthetic during their entire life cycle [e.g., the Western Australian fully

subterranean *Rhizanthella gardneri* (Batty et al. 2004)], the fungus continues to play this role. In all orchids, including those that are green (photosynthetic) as adults, the fungi also absorb mineral nutrients from soil (like AM, see below) (Cameron et al. 2006).

In ericoid mycorrhizas, a large number of infection points are found: up to 200 per mm root in *Calluna*, as opposed to 2–10 per mm in *Festuca ovina* (sheep's fescue), infected by an AM fungus. Up to 80% of the volume of these mycorrhizas may be fungal tissue (not including the external mycelium). The fungi infecting Ericaceae are Ascomycota (e.g., *Hymenoscyphus ericae*) (Cairney & Ashford 2002).

Spores of ectomycorrhizal fungi in the rhizosphere may germinate to form a **monokaryotic mycelium**. This fuses with another hypha, forming a **dikaryotic mycelium**, which can then colonize the root, forming a mantle of fungal hyphae that enclose the root. The hyphae usually penetrate intercellularly into the cortex, where they form the **Hartig net** (Fig. 3; Massicotte et al. 1999). The hyphae always remain apoplastic and can colonize the epidermal (angiosperms) and the cortical (gymnosperms) layers. As hyphae contact the root surface, roots may respond by increasing their diameter and switching from apical growth to precocious branching (Peterson & Bonfante 1994). Fungal biomass constitutes about 40% of ectomycorrhizas. Numerous fungal species have the capacity to form ectomycorrhizas. Most of these belong to the Basidiomycota and Ascomycota, and they are often species that we are familiar with as toadstools. Some of these are edible (e.g., *Boletus*, truffles), whereas others are highly toxic (e.g., *Amanita*).

In contrast to infection by pathogenic fungi, colonization with mycorrhizal fungi never causes disease symptoms. In the presence of mycorrhizal fungi in the rhizosphere, flavonoids accumulate in the roots of *Medicago sativa* (alfalfa) host roots, similar to, but much weaker than, the response to pathogenic fungal attack (Sect. 3 of Chapter 9C on effects of microbial pathogens). AM fungi initiate a transient **host defense response** in the early stages of colonization, followed by suppression to levels well below those of noncolonized plants. The production of defense-related gene products is restricted to arbusculated cells; intercellular hyphae and vesicles elicit no such defense response (Harrison & Dixon 1994, Harrison 1999, Hause & Fester 2005). The rate and location of fungal growth within the root may be controlled through activation of plant defense mechanisms.

Some mutants of *Pisum sativum* (pea) and other legumes are characterized by aborted mycorrhizal infections, after formation of appressoria (Duc et al. 1989, Shirtliffe & Vessey 1996). The genes appear to be linked with genes that control nodulation by rhizobia (Sect. 3.3) which may point to a tight control of two carbon-consuming and potentially competing symbioses (Sect. 2.6).

2.1.2 Mycorrhizal Responsiveness

In soils with low P availability, plants vary widely in the extent to which their growth responds to root colonization by mycorrhizal fungi (Johnson et al. 1997). **Mycorrhizal dependency** is the ratio of the dry mass of mycorrhizal plants to that of nonmycorrhizal plants. Species that depend less on AM fungi for their nutrient acquisition are generally colonized to a lesser extent in the field than more AM-dependent ones. This suggests that species that have root systems with low dependency on mycorrhizas also have mechanisms to suppress mycorrhizal colonization (Sect. 2.3.1). Mycorrhizal dependency of a plant species varies with the AM fungal species involved in the symbiosis (Van der Heijden et al. 1998a). In grassland communities, dominant species tend to have a greater mycorrhizal dependency than subordinate species, so that suppression of the AM symbiosis enhances plant species diversity (Hartnett & Wilson 2002). In other herbaceous communities, mycorrhizas enhance plant species diversity by increasing the establishment and abundance of subordinate species relative to the community dominants, and plant diversity may be positively correlated with the species diversity of mycorrhizal fungi (Van der Heijden et al. 1998b, O'Connor et al. 2002).

Plants that show little responsiveness to AM fungi in terms of growth, may, in fact, acquire significant amounts of P via the fungus (Smith et al. 2003, 2004, Li et al. 2006). Using a compartmented pot system and ^{33}P-labeled P_i (Fig. 5A), the contribution of the mycorrhizal uptake pathway to total plant P_i uptake can be estimated. The hyphal compartment is capped with 25 μm nylon mesh, which allows hyphae to penetrate, but excludes roots. Unlabeled P can be absorbed directly by roots or via the mycorrhizal pathway. Compared with noninoculated plants without additional P, *Linum usitatissimum* (flax) grows better, but to different extents, depending on the AM fungi tested (*Gigaspora rosea*, *Glomus caledonium*, or *Glomus intraradices*). *Medicago truncatula* (medic) responds positively to the two *Glomus* species in terms of dry weight production, but shows a small growth depression with *Gigaspora rosea*, compared with nonmycorrhizal plants. *Solanum lycopersicum* (tomato) does not respond positively to any of the fungi. P uptake also varies among the different plant–fungus combinations, and **mycorrhizal phosphorus dependencies**

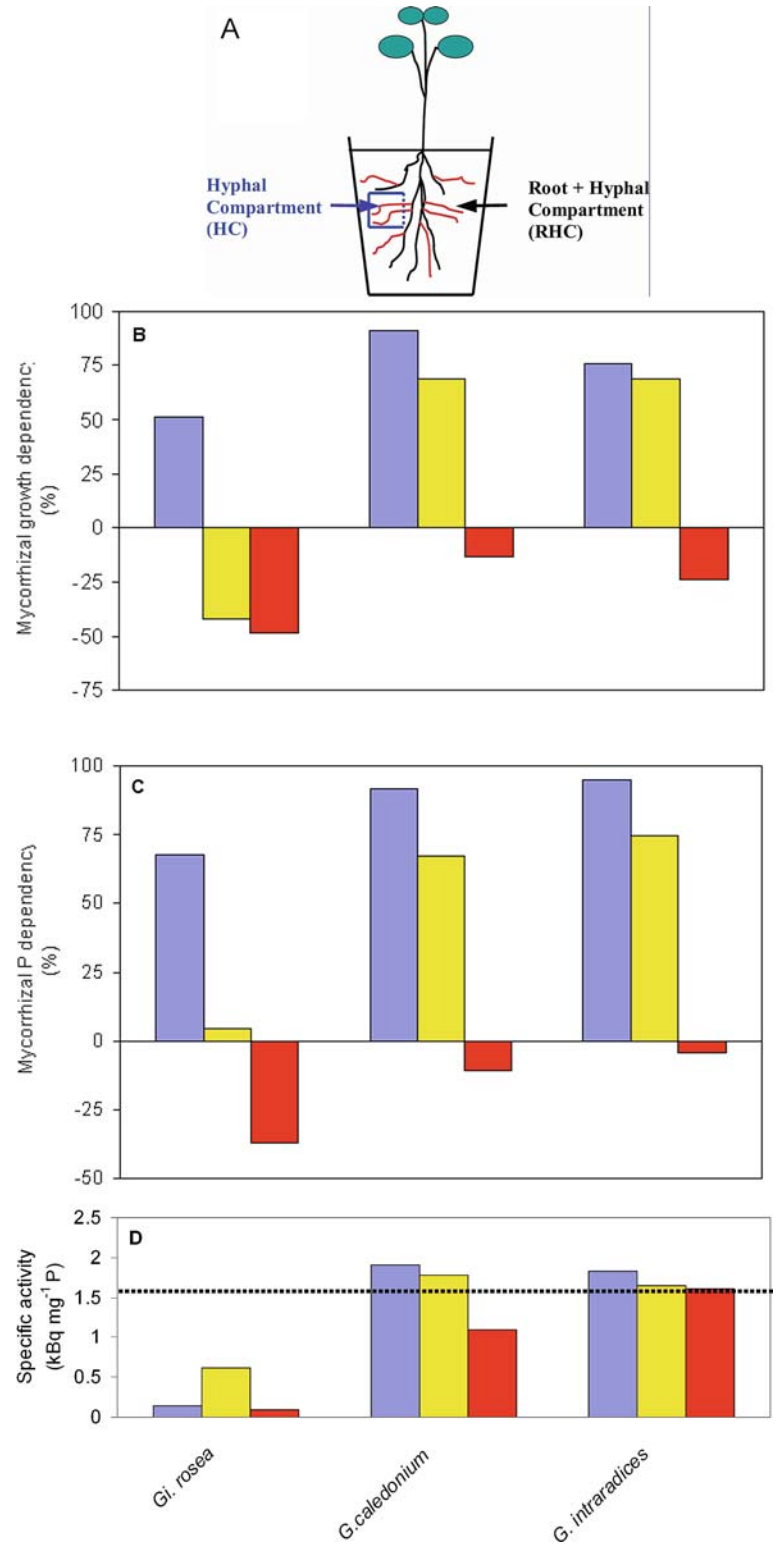


FIGURE 5. (A) Diagrammatic representation (not to scale) of a compartmented pot design to assess P uptake by mycorrhizas and roots. The main root + hyphae compartment is a nondraining pot containing a mixture of sand and soil. For mycorrhizal treatments, this mix includes inoculum of three appropriate fungi: *Gigaspora rosea*, *Glomus caledonium*, and *Glomus intraradices*. Nonmycorrhizal treatments receive no inoculum. The hyphal compartment is a small plastic tube containing the same soil + sand mix, but without inoculum; it is capped with 25 μm nylon mesh, which allows hyphae (shown in red) but not roots (shown in black) to grow into the hyphae compartment. The soil in the hyphae compartment is well mixed with ^{33}P-labeled orthophosphate of high specific activity. (B–D) Mycorrhizal effects on (A) growth, (B) total P uptake, and (C) specific activities of ^{33}P, in *Linum usitatissimum* (flax, blue bars), *Medicago truncatula* (medic, yellow bars), and *Solanum lycopersicum* (tomato, red bars). Mycorrhizal dependencies for growth and P-uptake are calculated as: 100 (value for mycorrhizal plant – mean value for nonmycorrhizal plants)/value for mycorrhizal plant. In the bottom panel, the dotted horizontal line indicates the predicted specific activity of ^{33}P in the plants if 100% of P is derived via the mycorrhizal pathway. This percentage is calculated using values for specific activities of ^{33}P in the plants and bicarbonate-extractable P in the hyphae compartment and the total P available in the pots and in the hyphae compartment. It assumes that the densities of hyphae (meters per gram of soil) are the same in the hyphae compartment and in the hyphae + roots compartment (after Smith et al. 2003). Copyright American Society of Plant Biologists.

(Fig. 5B, middle) are similar to **mycorrhizal growth dependencies** (Fig. 5B, top). By supplying[33] P in a compartment to which only the fungal hyphae has access (Fig. 5A), it is possible to show that the mycorrhizal pathway differs in its contribution to total P uptake, depending on fungal and plant species. P transfer via the mycorrhizal pathway is extremely high in five out of the nine individual plant–fungus combinations, although this is not correlated with mycorrhizal P dependency. With *Glomus intraradices* as the fungal partner, all of the P is delivered via the mycorrhizal pathway to all tested plants (Fig. 5B, bottom). These findings indicate that mycorrhizas may be an important pathway of P_i uptake, even in plants that lack a positive change in growth or P status as a result of AM colonization. It would be interesting to learn more about the conditions that cause down-regulation of P_i transporters responsible for P_i uptake from the root environment (Sect. 2.2.2 of Chapter 6 on mineral nutrition) when AM fungi infect the roots (Karandashov & Bucher 2005).

Mycorrhizal responsiveness is generally assessed using single species in a pot experiment, which poorly reflects the real world. When paired with a near-isogenic nonmycorrhizal genotype, even *Solanum lycopersicum* (tomato) shows a positive growth response (Cavagnaro et al. 2004), when this is not the case when tested singly (Fig. 5B, top). This aspect is further discussed in Sect. 2.2.

Crop cultivars, e.g., of *Zea mays* (corn) that have been developed for high-input systems in Europe have not lost their ability to be colonized, and may be more responsive to inoculation by *Glomus intraradices* than those suited for low-input African systems. However, specific adaptations that allow nonmycorrhizal plants developed for low-input systems to perform well in low-P soils may limit their ability to respond to higher nutrient supply rates and mycorrhizal infection. High-input cultivars may have traits that are useful for low-input cropping systems where mycorrhizal symbioses are established (Wright et al. 2005). A high mycorrhizal responsiveness is commonly associated with lack of well-developed root hairs and coarse fibrous roots (Sect. 2.2.1 of Chapter 6 on mineral nutrition; Collier et al. 2003).

2.2 Nonmycorrhizal Species and Their Interactions with Mycorrhizal Species

Although mycorrhizal associations are very common, some species cannot be colonized, or only marginally so (Brundrett 2002). These **nonmycorrhizal species** can be broadly categorized as either **ruderal** species that inhabit relatively fertile sites or species that occur on severely P-impoverished soils. The nonmycorrhizal ruderals include many that belong to Brassicaceae, Caryophyllaceae, Chenopodiaceae, and Urticaceae. The species from severely **P-impoverished habitats** include Cyperaceae, Proteaceae, and Restionaceae, as well as carnivorous (Chapter 9F) and parasitic species (Chapter 9D). The "scavenging" strategy of mycorrhizal species, which access P that is in the soil solution, but too far away from roots or inside soil pores that are too small for roots to enter, does not work on severely P-impoverished soils. The little amount of P that is present in these soils is predominantly sorbed to soil particles. Cluster roots, which release large amounts of carboxylates in an exudative burst (Sect. 2.2.5.2 of Chapter 6 on mineral nutrition) effectively "mine" P from these soils (Fig. 6). In younger landscapes, nonmycorrhizal species with cluster roots tend to occur on either

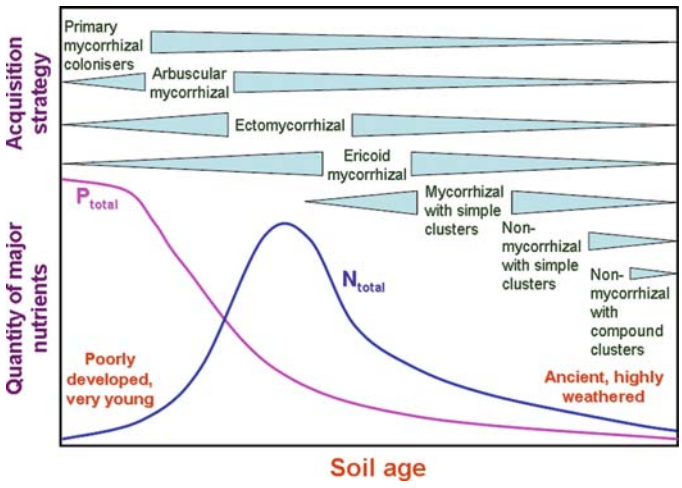


FIGURE 6. Changes in total soil P and N and in plant nutrient-acquisition strategies (green) as dependent on soil age. "Poorly developed, very young soils" refers to soils that result from, e.g., recent volcanic eruptions or glaciation; "ancient, highly weathered soils" refers to soils that have been above sea level and not been glaciated for millions of years. Whilst never becoming dominant in severely P-impoverished soils, some mycorrhizal species do co-occur with nonmycorrhizal, cluster-bearing species. The width of the triangles referring to the different ecological strategies provides a measure of the abundance of these strategies as dependent on soil age (modified after Lambers et al. 2008). Copyright Elsevier Science, Ltd.

calcareous soils, where the availability of P is low due to precipitation as calcium phosphates, or on acids soils, where P precipitates as iron or aluminum phosphates (Fig. 6.1 in Sect. 2.1 of Chapter 6 on mineral nutrition; Lambers et al. 2006, 2008).

Even within typical nonmycorrhizal genera, mycorrhizal infection has been observed in some species (Muthukumar et al. 2004, Boulet & Lambers 2005). It is interesting, as discussed in Sect. 2.2.5.2 of Chapter 6 on mineral nutrition, that many of the nonmycorrhizal species from severely P-impoverished soils have "**cluster roots**" (Cyperaceae, Proteaceae, and Restionaceae). Other nonmycorrhizal species include **carnivorous** species, e.g., *Drosera* (sundew), and **hemiparasitic** species, e.g., *Nuytsia floribunda* (Western Australian Christmas tree).

The mechanisms that prevent colonization in nonmycorrhizal species are not yet fully understood. In some species, the exudation of fungi-toxic compounds, such as **glucosinolates** in Brassicaceae (Koide & Schreiner 1992) or a chitin-binding **agglutinin** in *Urtica dioica* (stinging nettle) (Vierheilig et al. 1996), may prevent infection. More importantly, the correct chemical cues necessary for development after spores have germinated (Sect. 2.2.1) may be lacking (Harrison 2005).

Mycorrhizal fungi may enhance growth of mycorrhizal plants, at least at a low P supply. In some nonmycorrhizal species, however, the opposite is found (Sanders & Koide 1994). In these species, the mycorrhizal fungus may cause localized lesions on the roots around aborted penetration points (Allen et al. 1989), inhibit root-hair elongation, probably via its exudates, and reduce stomatal conductance (Allen & Allen 1984). This mechanism may well explain why nonmycorrhizal species show poor growth in a community dominated by mycorrhizal species, unless the P level is increased (Francis & Read 1994).

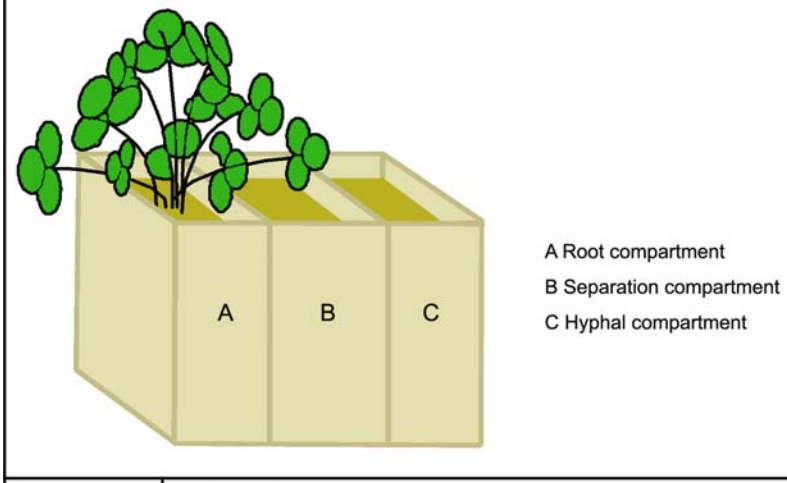
2.3 Phosphate Relations

Like root hairs, the external mycelium of mycorrhizas increases the roots' absorptive surface. In fact, the effective root length of the mycorrhizal associations may increase 100-fold or more per unit root length (Table 1).

2.3.1 Mechanisms That Account for Enhanced Phosphate Absorption by Mycorrhizal Plants

Arbuscular mycorrhizal associations enhance P uptake and growth most strongly, when the availability of P in the soil is fairly low (Sect. 2.2.5.2 of Chapter 6 on mineral nutrition; Bolan et al. 1987, Thingstrup et al. 1998). Experiments using different soil compartments and different labeled sources of P_i show that AM plants do not have access to different chemical pools of P_i in soil (Fig. 7). They are capable, however, of acquiring P outside the depletion zone that surrounds the root, because of the **widely ramified hyphae**. These hyphae allow P transport over as much as 10 cm from the root surface and at rates that far exceed diffusion in soil (Fig. 8). In addition, they may access smaller soil pores and compete effectively with other microorganisms (Joner & Jakobsen 1995); some AM may access organic P, but the ecological significance of this remains to be established (Joner et al. 2000a,b, Koide & Kabir 2000). Ectomycorrhizal hyphae can extend even greater distances, possibly several meters. Ectomycorrhizal and ericoid mycorrhizal roots also have access to additional chemical pools of P; they may release **phosphatases**, which enhance the availability of organic P, or exude **carboxylates**, which increase the availability of sparingly soluble P (Landeweert et al. 2001, Van Leerdam et al. 2001, Van Hees et al. 2006). *Hymenoscyphus ericae*, which forms mycorrhizas with a number of host plants belonging to the Ericaceae, also produces extracellular enzymes that may allow the fungus to decompose components of plant cell walls which facilitates access to mineral nutrients that are sequestered in these walls (Leake & Read 1989, Read 1996, Cairney & Burke 1998). *Laccaria bicolor*, an ectomycorrhizal fungus, is capable of paralyzing, killing, and digesting **springtails**. A significant portion of the organic N that is subsequently hydrolyzed ends up in its macrosymbiont, *Pinus strobus* (eastern white pine) (Klironomos & Hart 2001). Springtails selectively feed on fungi, including mycorrhizal fungi. Should this phenomenon prove to be widespread, forest–nutrient cycling may turn out to be more complicated and tightly linked than is currently recognized. Interestingly, springtails prefer nonmycorrhizal species when given a choice; their fecundity is reduced when fed a diet of less palatable mycorrhizal fungi (Klironomos et al. 1999).

Ectomycorrhizas and ericoid mycorrhizas frequently occur in organic soils, whereas AMs are more typical of mineral soils. Weatherable minerals under many European coniferous forests contain a dense network of tubular micropores with a diameter of 3–10 μm that are formed by carboxylates exuded by mycorrhizal or saprotrophic fungi. Concentrations of succinate, oxalate, formate, citrate, and malate may be in the micromolar to millimolar range. These exuded carboxylates enhance mineral



P source	Shoot ^{32}P content as a percentage of total P uptake (%)	
	Nonmycorrhizal	Mycorrhizal
Ca_2-P	0.2	18.5
Ca_8-P	0.9	13.2
$Ca_{10}P$	0.7	0.8
A1-P	1.2	14.0

FIGURE 7. Diagram showing the design of the rhizoboxes used to assess which chemical forms of P can be accessed by arbuscular mycorrhizal hyphae. The ^{32}P-labeled P source is added to the hyphal compartment only. The ^{32}P content is expressed as a proportion of the total P content of the shoots of *Trifolium pratense* (red clover) (Yao et al. 2001).

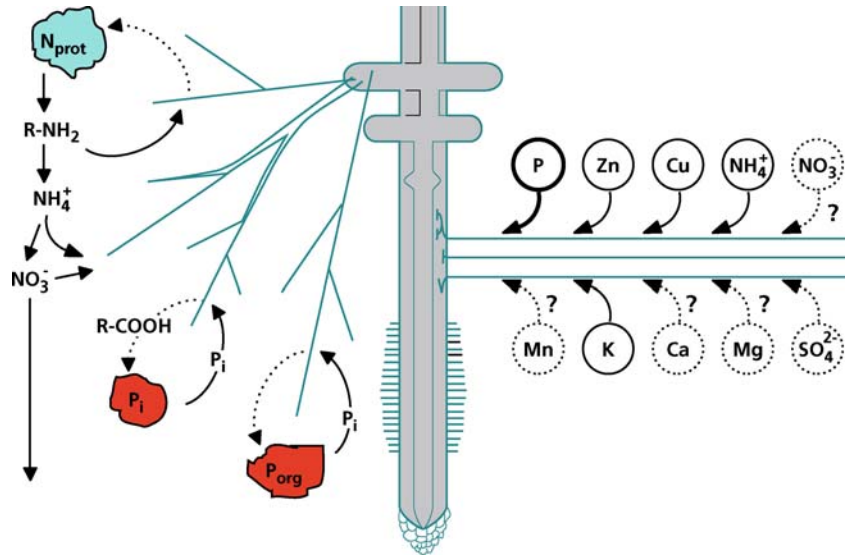


FIGURE 8. Schematic presentation of components of the nutrient acquisition from the soil by arbuscular mycorrhizal roots (*right*). The thickness of the circle indicates the importance of AM in acquiring this nutrient (? indicates lack of definitive information). Additional components in ectomycorrhizal roots are also shown (*left*). Note that all mycorrhizas enhance the availability of soil nutrients by enlarging the soil volume that is exploited, and that this is most relevant for those nutrients that are least mobile (e.g., phosphate). Ectomycorrhizas excrete hydrolytic enzymes, which allow them to use organic forms of both P and N, and chelating organic acids, which allows the use of poorly soluble forms of phosphate (after Marschner & Dell 1994).

weathering by forming complexes with Al and dissolve Ca-rich rock (Wallander 2000). In this way about 10^7 hyphal tips are eating their way through sand grains at any one time, forming 150 km of micropores per m^3 of soil per year. Ectomycorrhizal hyphae of the species *Suillus granulatus* and *Piloderma croceum* are thought to transport the released minerals directly to their hosts, thereby bypassing competition for nutrient uptake by other organisms. This mechanism by which mycorrhizal hyphae bypass the soil to reach minerals might help explain why forest productivity has not decreased, despite recent excessive soil acidification (Jongmans et al. 1997).

The external AM mycelium consists of both large "runner" hyphae and finer hyphae with a role in nutrient absorption. As in roots, P_i uptake by the fine mycorrhizal hyphae occurs via active transport, against an electrochemical potential gradient, with a proton-cotransport mechanism (Sect. 2.2.2 of Chapter 6 on mineral nutrition). Once absorbed by the external hyphae, P is rapidly transported into vacuoles where most of it is polymerized into inorganic **polyphosphate** (poly-P), a linear polymer of three to thousands of inorganic phosphate molecules, connected by high-energy phosphate bonds (Ezawa et al. 2002). This reaction is catalyzed by polyphosphate kinase, which is induced when excess P_i is absorbed. Poly-P accumulated in the vacuole is translocated from the external hyphae to the hyphae inside the roots, possibly via cytoplasmic streaming. This transport of P and that of other immobile ions through the external hyphae to the plant is relatively rapid, bypassing the very slow diffusion of these ions in soil. Once the poly-P has arrived near the plant cells, it is degraded, presumably catalyzed by **phosphatases**. The mechanisms accounting for poly-P production at one end of the hyphae and breakdown at the other are unknown. It might involve sensing a gradient in plant-derived carbohydrates. Transfer of P and other nutrients from fungus to plant is a two-step process over the membranes of the two symbionts, probably involving passive efflux from the fungus and active uptake by the plant (Javot et al. 2007). Some of the plant P_i-transporter genes involved in this process are mycorrhiza-specific and differ genetically from those discussed in Sect. 2.2.2 of Chapter 6 on mineral nutrition (Karandashov & Bucher 2005). These mycorrhiza-inducible P_i-transporter genes are upregulated in roots that are colonized by AM fungi and expressed at a very low level in noncolonized parts of the same root system. These findings show that in species that form AM associations with members of the Glomeromycota P_i transporters have evolved that are involved in scavenging P from the apoplast between intracellular AM structures and root cortical cells (Rausch et al. 2001, Glassop et al. 2005).

2.3.2 Suppression of Colonization at High Phosphate Availability

The AM fungus colonizes roots to a greater extent in low-P soils than in soils that are more fertile (Fig. 9; Smith & Read 2008). To some extent this greater

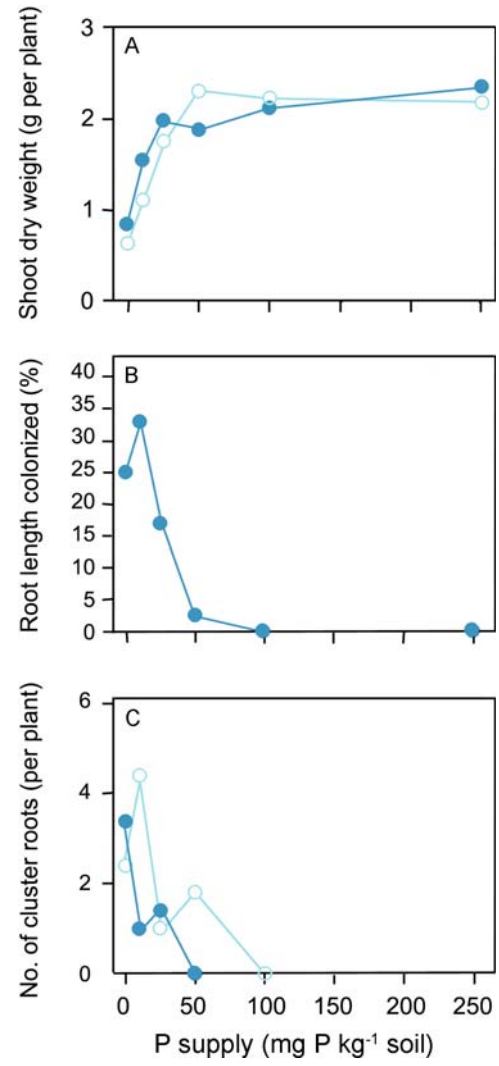


Figure 9. Response of *Casuarina cunninghamiana* (sheoak) seedlings to inoculation with an arbuscular mycorrhizal fungus (*Glomus* sp.) over a range of P supplies in sand culture. (A) Shoot dry weight; (B) mycorrhizal colonization of roots; (C) occurrence of cluster roots. Filled symbols: inoculated with *Glomus*; open symbols uninoculated (redrawn after Reddell et al. 1997, *Australian Journal of Botany* 45: 41–51, Copyright CSIRO, Australia).

frequency may be associated with a decreased rate of root elongation so that the colonization by the fungus keeps up with the growth of the root. **Systemic effects** of P are also important, however. An analysis of *Solanum tuberosum* (potato) grown with a divided root system of which only one half is inoculated with the AM fungus *Glomus intraradices* shows that when high P levels are applied to the noncolonized part of the root system, the formation of both arbuscules and vesicles is suppressed in the colonized portion of the root, despite the presence of more internal hyphae. Moreover, a high P_i supply may lead to down-regulation of the expression level of both the mycorrhiza-inducible and other P_i transporters (Rausch et al. 2001, Glassop et al. 2005).

Once a root is infected by an AM fungus, further infection of the roots tends to be suppressed (Vierheilig et al. 2000); this phenomenon is called **autoregulation**. A decreased availability of carbohydrates or an improved P status of the plant has been offered as an explanation for this suppression of further colonization. To test this, plants of *Hordeum vulgare* (barley) were grown with a divided root system, where one half is inoculated with an AM fungus. After extensive root colonization, the other half is inoculated, but colonization is suppressed (Fig. 10). In such plants some of the P acquired by the colonized root part ends up in the roots that are not colonized, as a result of transport via the xylem to the shoot, followed by export via the phloem from the shoot. Because the biomass and the P concentration of both root halves are the same, neither a shortage of carbohydrates nor elevated P concentrations explain the suppression (Fig. 10). A **systemic suppression**, i.e., a response triggered by mycorrhizal colonization followed by signaling to the rest of the plant, is the most likely explanation for the autoregulatory effect of prior mycorrhization on subsequent colonization. This involves signaling molecules such as **strigolactones** (Sect. 2.1.1), whose release from roots of plants that are hosts for AM fungi is promoted by P deficiency (Yoneyama et al. 2007a). By contrast, in roots of nonmycorrhizal species, P deficiency does not affect exudation (Yoneyama et al. 2007b). Interestingly, the systemic autoregulatory effect not only suppresses mycorrhization in *Hordeum vulgare* (barley), but also reduces root **infection** by the take-all **disease** caused by the fungus *Gaeumannomyces graminis* var. *tritici*. This effect is found when barley plants show a high degree of mycorrhizal root colonization, whereas a low mycorrhizal root colonization has no effect on take-all (Khaosaad et al. 2007).

2.4 Effects on Nitrogen Nutrition

Unlike AM, some **ectomycorrhizas** may utilize **organic N**, including proteins. This has been documented in situ, as revealed by a comparison of **^{15}N fractionation** in plants with and without ectomycorrhiza. Ectomycorrhizal plants may have a 1.0–2.5‰ more positive $\delta^{15}N$ value than do plants infected with arbuscular mycorrhiza (Table 2), demonstrating that different N sources are used (either different compounds or different regions in the soil). A study on a Tanzanian woodland (Table 2) provides no evidence for a difference in isotope composition in different soil layers. Fractionation of the heavy N isotope (^{15}N) occurs during **mineralization** and **nitrification**; therefore, the organic N becomes enriched with ^{15}N. The data in Table 2 provide evidence that the ectomycorrhizal plants use a significant amount of N from a pool that was not decomposed and nitrified (i.e., organic N). In boreal forest and arctic tundra, ectomycorrhizal plant species also have distinctive ^{15}N signatures, with ^{15}N concentrations that are higher than those of species with ericoid mycorrhizas, but lower than those of AM or nonmycorrhizal species (Schulze et al. 1995, Nadelhoffer et al. 1996). In these studies either rooting depth or the form of N that is utilized could have contributed to the different ^{15}N signatures.

Ericoid mycorrhizas, like ectomycorrhizas, can use quite **complex organic sources of N and P** (Fig. 8; Read 1996). This ability may contribute to the dominance by Ericaceae of many cold and wet soils, where rates of decomposition and mineralization are low (Read & Perez-Moreno 2003). AMs are at the other extreme of the continuum of mycorrhizal associations. They cannot access organic P, because of a very low capacity to release phosphatases into the soil (Joner et al. 2000a). Their predominant significance lies in the acquisition of sparingly available inorganic nutrients, especially P. AMs are relatively unimportant for acquisition of N, if this is available as NO_3^-, but they do enhance N acquisition when mineral N is present as the less mobile NH_4^+ (Johansen et al. 1994, Tanaka & Yano 2005). In the extraradical hyphae, ammonium is assimilated into **arginine**, and then transported toward the arbuscules, where arginine is broken down, followed by transfer of NH_4^+ to the host cells (Govindarajulu et al. 2005, Jin et al. 2005, Chalot et al. 2006). AM may enhance the uptake of NO_3^- from dry soils, when mass flow and diffusion are limited, but not in wet soils (Tobar et al. 1994). Ectomycorrhizas are thought to be intermediate between

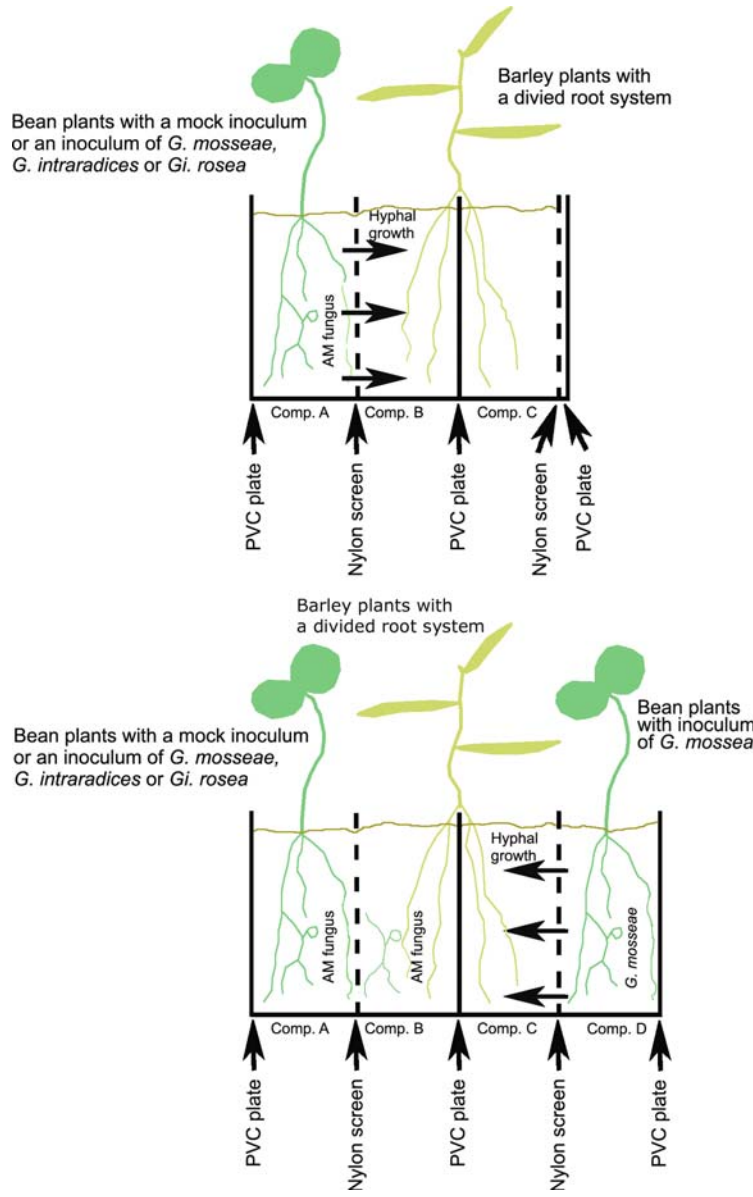


FIGURE 10. Diagram showing the experimental design of the rhizoboxes used to assess the systemic effect of prior infection of *Hordeum vulgare* (barley) by arbuscular mycorrhizal fungi (compartment B) on subsequent infection (in compartment C). Infection takes place from roots of *Phaseolus vulgaris* (common bean) in adjacent compartments (A or D) (modified after Vierheilig et al. 2000).

the ericoid and arbuscular mycorrhiza in terms of accessing organic N (Lambers et al. 2008).

2.5 Effects on the Acquisition of Water

Arbuscular mycorrhizal plants may have an enhanced capacity to acquire water from the root environment (Augé 2001). Several hypotheses have been put forward to explain this increased capacity. One suggests an indirect effect via the **improved P status** of the plant which increases the hydraulic conductance of the roots or affects the plant's hormone metabolism and stomatal conductance. *Lactuca sativa* (lettuce) plants colonized by the AM fungi *Glomus coronatum*, *Glomus intraradices*, *Glomus claroideum*, and *Glomus mosseae* deplete soil water to a greater extent than uninoculated control plants or plants colonized by *Glomus constrictum* or *Glomus geosporum*. The differences in soil-moisture depletion can be ascribed to the activity of AM fungi, but fungi differ in their effectiveness to enhance plant water uptake from soil, probably related to the amount of external mycelium produced by each AM fungus

Table 2. ^{15}N abundance of leaf samples collected in different years in Tanzania.*

Species	Symbiotic status	$\delta^{15}N$ 1980	1981	1984
Brachystegia boehmii	EC	1.64	1.32	1.23
B. microphylla	EC	1.53	1.51	1.73
Julbernardia globiflora	EC	2.81	1.63	1.60
Pterocarpus angolensis	AM+NO	−0.81	−0.87	−0.93
Diplorynchus condylocarpon	AM	–	−0.36	−0.60
Xeroderris stuhlmannii	AM+NO	–	0.01	0.62
Dichrostachys cinerea	AM+NO	–	0.45	−0.38

Source: Högberg (1990).

* EC = ectomycorrhizal; AM = arbuscular mycorrhizal; NO = nodulated. The experiments summarized here were actually carried out with the aim to determine the extent of symbiotic N_2 fixation of the nodulated plants. Since nodulated plants have access to dinitrogen from the atmosphere, they are expected to have $\delta^{15}N‰$ values closer to atmospheric N_2 than do plants that do not fix dinitrogen. The data presented here stress that control plants need to be sampled to allow a proper comparison. This table shows that the choice of the control plants is highly critical (see also Sect. 3 of this chapter).

and to the frequency of root colonization in terms of live and active fungal structures (Marulanda et al. 2003). The multihyphal strands of ectomycorrhizas are thought to have a particularly high capacity to transport water. Alternatively, the improved plant water status may be an effect on effects of mycorrhizas on soil structure (Bearden & Petersen 2000).

2.6 Carbon Costs of the Mycorrhizal Symbiosis

Mycorrhizas provide nutritional benefits, which may enhance **resource allocation to leaves** (Baas & Lambers 1988, Grimoldi et al. 2005) and **photosynthetic carbon gain** of the host (Douds et al. 1988, Wright et al. 1998a), but they also incur **carbon costs** for the host. The amount of carbon that is exuded from intact roots into the apoplast and normally ends up in the rhizosphere is not sufficient to satisfy the demand of the microsymbiont of the mycorrhizal association. It is possible that passive efflux of carbon is increased in mycorrhizal plants; alternatively, the host's active carbon-uptake system may be inhibited. So far there is no molecular information on transport proteins that account for carbon efflux from or carbon re-uptake in mycorrhizal plants (Bago et al. 2000).

Costs associated with the AM symbiosis have been estimated in various ways (e.g., by comparing plants with and without the mycorrhizal symbiont at the same growth rate). This can be achieved by providing more P to the nonmycorrhizal plant, compared with the supply to the mycorrhizal plant. The carbon use for growth and respiration by the roots of both types of plants can then be used to quantify costs of the mycorrhizal symbiosis (Snellgrove et al. 1982, Grimoldi et al. 2006). The problem with this method is that it assumes steady-state rates of P acquisition and carbon consumption, whereas in fact these may vary following active root colonization. A variation of this approach is to grow nonmycorrhizal plants at a range of P supplies, so that a P–response curve can be constructed with which to compare the mycorrhizal plants (Rousseau & Reid 1991, Eissenstat et al. 1993).

An alternative approach to quantify the costs of the mycorrhizal symbiosis has been to grow plants with a divided root system. That is, part of the root system is grown in one pot, and the remaining part in a separate pot. One part of the divided root is then inoculated with a mycorrhizal fungus, while the other is not and remains nonmycorrhizal. The shoot is then given $^{14}CO_2$ to assimilate in photosynthesis and the partitioning of the label over the two root parts is measured (Table 3; Koch & Johnson 1984, Douds et al. 1988). It is also possible to calculate carbon costs of the mycorrhizal symbiosis by measuring the flow of ^{14}C-labeled assimilates into soil and external hyphae (Jakobsen & Rosendahl 1990).

The estimates of the carbon costs of the AM symbiosis vary between 4 and 20% of the carbon fixed in photosynthesis (Lambers et al. 2002). Only a minor part (15%) of the increased rate of root respiration is associated with an increased rate of ion uptake by the mycorrhizal roots. The major part (83%) is explained by the respiratory metabolism of the fungus and/or other effects of the fungus on the roots' metabolism (Baas et al. 1989). Construction costs of fibrous roots

Table 3. Comparison of accumulated ^{14}C and fresh mass in mycorrhizal and nonmycorrhizal halves of root system of two citrus cultivars.*

	^{14}C recovered from below-ground tissue dpm g^{-1}		Fresh mass mg $plant^{-1}$	
Species	+	−	+	−
Sour orange	66.4	33.6	1580	1240NS
Carrizo citrange	67.7	32.3	1990	1520NS

***Source*: Koch & Johnson (1984).**
*+ and − denote mycorrhizal and nonmycorrhizal plants, respectively; NS indicates that there was no significant difference.

are also higher for mycorrhizal than for nonmycorrhizal roots, because of their higher fatty acid concentration (Sect. 5.2.1 of Chapter 2B on plant respiration; Peng et al. 1993). The costs for the ECM symbiosis are probably higher, but there is little information available (Hobbie 2006).

In addition to a higher carbon expenditure, mycorrhizal plants also tend to have a higher rate of photosynthesis per plant, partly due to higher rates of photosynthesis per unit leaf area and partly due to their greater leaf area (Wright et al. 1998a, b). The higher rate of CO_2 assimilation is most pronounced when the soil water potential is low (Sanchez-Diaz et al. 1990). When P and water are limiting for growth, therefore, benefits outweigh the costs, and mycorrhizal plants usually grow faster, despite the large carbon sink of the symbiotic system. The relatively high costs of the mycorrhizal association, however, may help to explain why mycorrhizal plants sometimes grow less than their nonmycorrhizal counterparts (Thompson et al. 1986, Fredeen & Terry 1988), especially when a second microsymbiont (rhizobium) plays a role (Fig. 11). Under drought, however, mycorrhizal plants may still show more benefit from an association with rhizobium than do nonmycorrhizal control plants (Penas et al. 1988).

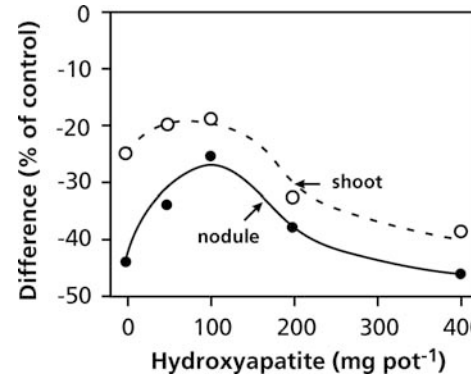


Figure 11. The relative host response to mycorrhizal infection as dependent on the supply of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. *Phaseolus vulgaris* (common bean) plants are infected with *Rhizobium phaseoli*, a nitrogen-fixing bacteria. Half of the plants are also infected with the mycorrhizal fungus *Glomus fasciculatum*. The difference in mass of the parts of the mycorrhizal plants relative to the nonmycorrhizal control plants is calculated as percentage of the difference. Negative values indicate that the shoot or nodule mass is less in the mycorrhizal plants (after Bethlenfalvay et al. 1982). Copyright American Society of Plant Biologists.

2.7 Agricultural and Ecological Perspectives

From an ecological point of view, information on the mycorrhizal status of plants in a community is most important. In a mixed community **nonmycorrhizal species** may profit most from fertilization with P because the mycorrhizal association is often suppressed at a higher P supply, and not necessarily because the growth of nonmycorrhizal species is more severely P-limited (Sect. 2.3). Suppression of the mycorrhizas might then reduce the harmful effect of the mycorrhizal fungus on nonmycorrhizal species (Sect. 2.2). We should therefore be cautious in interpreting the effects of P fertilization on the growth of certain plants in a community.

Close proximity between the roots of a seedling and those of an established, infected plant may speed up AM infection, but, for unknown reasons, this is not always the case (Newman et al. 1992). AMs may have profound effects on interactions between plants in a community, as discussed in Sect. 7 of Chapter 9E on interactions among plants.

Mycorrhizas can obviously never enhance growth and productivity of crop plants in the absence of any P. Mycorrhizal associations,

however, do have great potential in improving crop production when P or other immobile nutrients are in short supply. Introduction of spores of the best microsymbiont and breeding for genotypes with a more efficient mycorrhizal symbiosis are tools that can be used to enhance food production in countries where immobile nutrients restrict crop production. As such, mycorrhizas allow good crop growth and

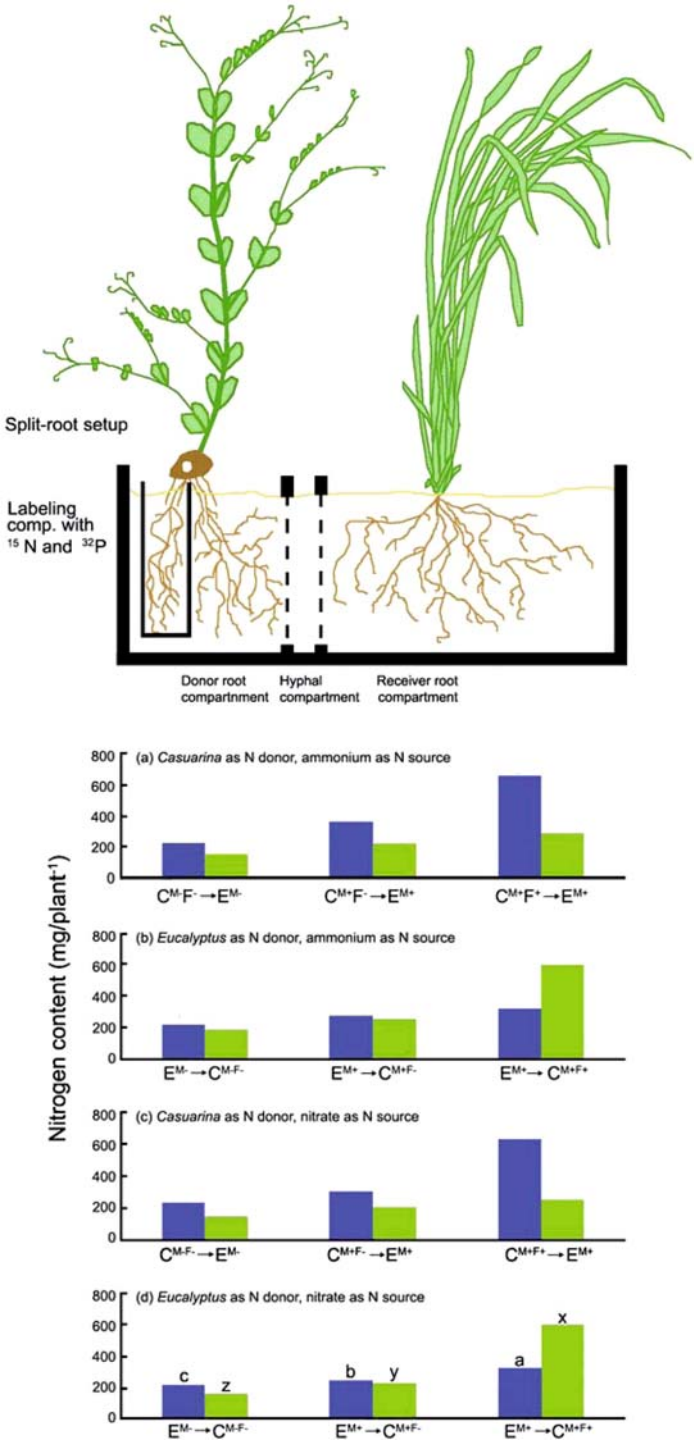


FIGURE 12. (*Top*) Planting arrangement in a split-root design. (*Bottom*) N content of *Casuarina cunninghamiana* and *Eucalyptus maculata* seedlings (blue bars for donors and green bars for receivers) as affected by mycorrhizas, nodulation, N source, and identity of the N-donor or N-receiver. Plants were fertilized with ^{14}N for 5 months and with ^{15}N (N donor only) for 1 month before harvest. Abbreviations: C, *Casuarina cunninghamiana*; E, *Eucalyptus maculata*; M, mycorrhizal status; F, nodulation status (He et al. 2004). Copyright Trustees of The New Phytologist.

may reduce nutrient losses to the surrounding environment (Tisdall 1994).

Arbuscular and ectomycorrhizal mycorrhizal fungi may infect many plants at the same time, even plants of different species, potentially providing a pathway for transport of carbon or nutrients between roots of different plants. Two-way N transfers mediated by a **common mycorrhizal network** have been examined by growing plants in two-chambered pots separated by nylon mesh, and supplying $^{15}NH_4^+$ or $^{15}NO_3^-$; the mesh effectively excludes root contact, but allows movement of water-soluble substances and penetration of fungal hyphae (Johansen & Jensen 1996). Using this method, it can be demonstrated that nitrogenous compounds are transported bidirectionally between N_2-fixing *Casuarina cunninghamiana* trees (sheoak) and *Eucalyptus maculata* trees (spotted gum), connected via the ectomycorrhizal fungus *Pisolithus* sp. (Fig. 12), especially in the nodulated, ectomycorrhizal treatment. About twice as much N moves from *Eucalyptus maculata* toward *Casuarina cunninghamiana* as in the opposite direction, irrespective of the source of N ($^{15}NH_4^+$ or $^{15}NO_3^-$), resulting in increased growth of *Casuarina cunninghamiana* due to interspecific N transfer. Since there is virtually no N transfer in the nonmycorrhizal treatment, but significant N transfer in the mycorrhizal treatment, N transfer between the two tree species is obviously mediated by ectomycorrhizal fungi. The much higher N transfer between nodulated mycorrhizal plants indicates that mycorrhizas and *Frankia* together enhance bidirectional N fluxes between N_2-fixing *Casuarina cunninghamiana* and non-N_2-fixing mycorrhizal *Eucalyptus maculata*, contradicting the view that N flows from N_2-fixing to non-N_2-fixing plants. However, the fraction of N derived from transfer is similar for both species, because the N concentrations are higher in the N_2-fixing *Casuarina cunninghamiana* (He et al. 2004, 2005). It remains to be established how significant bidirectional transport of nutrients or carbon is in the field, especially via AM networks (Pfeffer et al. 2004). Using stable carbon isotopes to assess carbon transfer via AM, provides no evidence for carbon transfer from *Festuca idahoensis* (Idaho fescue) to the exotic invasive *Centaurea maculosa* (spotted knapweed). However, *Centaurea maculosa* exploits its mycorrhizal symbiosis more effectively than the native grassland species, probably due to the luxury consumption of P through efficient utilization of extra-radical hyphae. Exploitation of AM networks may be a mechanism by which **invasive weeds** outcompete their neighbors (Zabinskey et al. 2002). Transport of carbon via mycorrhizal hyphae is obviously also important in **mycoheterotrophic** plants, which depends on carbon supplied by a photosynthetic plant to which they are connected via mycorrhizal hyphae (Sect. 2.1.1; Selosse et al. 2006). However, putatively autotrophic orchids also receive significant amounts of carbon from their fungal associates (Gebauer & Meyer 2003).

Benefits of N nutrition to non-N_2-fixing plants by neighboring N_2-fixing plants are well documented (Sect. 3.6). Differences in net N transfer with different nodulation/mycorrhizal combinations could have important ecological implications, both for nutrient cycling and for the structure and function of natural or agricultural plant communities, particularly with respect to those plants that can potentially construct a common mycorrhizal network to transfer nutrients (He et al. 2005). AM colonization can result in resource equalization or sharing, thus reducing dominance of aggressive species and promoting coexistence and biodiversity (Van der Heijden et al. 1998a). In tallgrass prairie, Fischer Walter et al. (1996) documented transfer of (labeled) P over distances of up to 0.5 m. Transfer of label, however, does not imply a net transfer of P. Although tracer experiments do show that interplant transfer does occur, the amount of transfer of ^{32}P between mycorrhizal plants of *Lolium perenne* and *Plantago lanceolata* appears to be much too slow to be ecologically significant (Eissenstat 1990).

3. Associations with Nitrogen-Fixing Organisms

Nitrogen is a major limiting nutrient for the growth of many plants in many environments in young landscapes (Sect. 2.1 of Chapter 6 on mineral nutrition). Terrestrial N is subject to rapid turnover, and is eventually lost as nitrogen gas into the atmosphere or deposited in marine sediments. Its maintenance, therefore, requires a continuous reduction of atmospheric N_2. Biological reduction of dinitrogen gas to ammonia can be performed only by some prokaryotes and is a highly O_2-sensitive process. The most efficient N_2-fixing microorganisms establish a symbiosis with higher plants, in which the energy for N_2 fixation and the O_2-protection system are provided by the plant partner (Mylona et al. 1995).

Symbiotic associations with microorganisms that fix atmospheric N_2 may be of major importance for a symbiotic plant's N acquisition, especially in environments where N is severely limiting to plant growth. As such, the symbiosis is also of agronomic and environmental importance, because it reduces the need for costly fertilizers and greenhouse gas emissions associated with their production.

A nonsymbiotic association [e.g., with *Azospirillum* in the rhizosphere of tropical grasses or *Gluconacetobacter diazotrophicus* in the apoplast of the stems of *Saccharum officinarum* (sugarcane)] is sometimes found. Contrary to the strictly symbiotic systems, no special morphological structures are induced.

Symbiotic N_2-fixing systems require a carbon input from the host that is far greater than the carbon requirements for the acquisition of N in the combined form (e.g., NO_3^-, NH_4^+, or amino acids). Are there mechanisms to suppress the symbiosis when there is plenty of combined N around? How does a plant discriminate between a symbiotic guest and a pathogenic microorganism? Finally, what is the significance of nonsymbiotic N_2 fixation for plants? To answer these ecological questions we will first provide a basic understanding of some physiological aspects of this symbiotic association.

3.1 Symbiotic N_2Fixation Is Restricted to a Fairly Limited Number of Plant Species

Because of its overwhelming economic importance, the most widely studied associations between N_2-fixing microorganisms and vascular plants are those

FIGURE 13. N_2-fixing symbiotic systems. (*Top, left*) Legume–rhizobium symbiosis on the South African *Chamaecrista mimosoides* (fishbone dwarf cassia) (photo H. Lambers). (*Bottom, left*) Symbiotic structure (rhizothamnia) between the Western Australian *Allocasuarina humilis* and an Actinobacteria (*Frankia*) (courtesy M.W. Shane, School of Plant Biology, The University of Western Australia, Australia). (*Right*) Symbiotic structure between *Macrozamia riedlii* and cyanobacteria (courtesy M.W. Shane, School of Plant Biology, The University of Western Australia, Crawley, Australia).

that involve a symbiosis between bacteria of the family Rhizobiaceae (genera: *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Rhizobium*, *Mesorhizobium*, and *Sinorhizobium*, collectively known as **rhizobia**; the numbers of genera are increasing steadily, and they include both alpha and beta Proteobacteria; Sprent & James 2007) and more than 3000 species of **Fabaceae** (Geurts & Bisseling 2002, Vessey et al. 2005). *Parasponia* is the only nonlegume species known to have a symbiotic association with rhizobium [*Bradyrhizobium* and *Rhizobium* (Vessey et al. 2005)]. Invariably, **root nodules** are formed (Figs. 13 and 14), with the exception of *Azorhizobium* and *Bradyrhizobium*, which induces nodules on both stems and roots (of *Sesbania rostrata* and *Aeschynomene* species, respectively). The Fabaceae family comprises three subfamilies; the less specialized subfamily Caesalpinioideae includes far more nonnodulating species than do the other two subfamilies (Van Rhijn & Vanderleyden 1995). The symbiosis between rhizobia and legume crops is of enormous agronomic importance, especially where fertilizer inputs are low.

There are also **nonlegume species** capable of forming a symbiotic association with N_2-fixing organisms, other than rhizobia. First, there is the **actinorhizal symbiosis** between soil bacteria (*Frankia*, Actinobacteria) and more than 200 species from eight nonlegume families of angiosperms [e.g., *Alnus* (alder), *Hippophae* (sea buckthorn), *Myrica* (myrtle), *Elaeagnus* (silverberry), *Dryas* (mountain avens), and *Casuarina* (sheoak)]. In all these symbioses **root nodules** are formed (Figs. 13 and 14; Akkermans & Hirsch 1997, Vessey et al. 2005). Second, there are symbioses between **Cyanobacteria** (*Nostoc*, *Anabaena*) and plant species of the genera *Azolla*, *Macrozamia*, and *Gunnera* (Vessey et al. 2005). Special morphological structures are sometimes formed on the roots (e.g., the **coralloid roots** in *Macrozamia* species) (Figs. 13 and 9.A.14; Pate et al. 1988). The endosymbiont only fixes N_2, not CO_2, although cyanobacteria are photosynthetically active when free-living (Lindblad et al. 1991). *Nostoc* in the *Gunnera–Nostoc* symbiosis maintains its photosystem II (PS II) units, but their photochemical efficiency is much reduced (Black & Osborne 2004). In the symbiosis between fungi of the genus *Collema* and cyanobacteria (*Nostoc*), the cyanobacteria are photosynthetically active; this symbiosis occurs in **lichens**.

Table 4 gives an overview of major symbiotic associations between plants and microorganisms

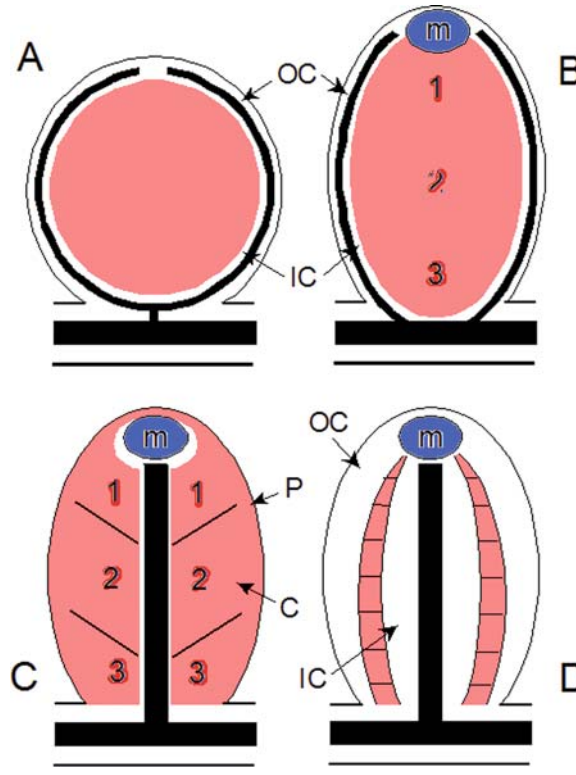


FIGURE 14. Diagrammatic representation of longitudinal sections through (A) an indeterminate legume nodule, (B) a determinate legume nodule, (C) an actinorhizal nodule, and (D) a lobe of a symbiotic coralloid root cluster. The red colored regions represent the infected zones. The dark, thick lines represent vascular tissues. Outer cortical (OC) tissue, inner cortical (IC) tissue, and meristems (m, blue) are indicated. In the indeterminate legume nodule (B) and the actinorhizal nodule (C), the zones of infection (1), N_2 fixation (2), and senescence (3) are indicated (Vessey et al. 2005).

Table 4. Symbiotic associations between plants and microorganisms capable of fixing atmospheric N_2.*

Plant type	Genus	Microorganism	Location	Amount of N_2 fixed (kg N ha^{-1} $season^{-1}$)
Fabaceae	*Pisum*	*Rhizobium*	Root nodules	10–350
	Glycine	*Bradyrhizobium*	Root nodules	15–250
	Medicago	*Sinorhizobium*	Root nodules	440–790
	Sesbania	*Azorhizobium* *Mesorhizobium*	Stem nodules	7–324
Ulmaceae	*Parasponia*	*Bradyrhizobium*	Root nodules	20–70
Betulaceae	*Alnus*	*Frankia*	Root nodules	15–300
Casuarinaceae	*Casuarina*	*(Actinobacteria)*	Root nodules	9–440
Eleagnaceae	*Eleagnus*	*(Actinobacteria)*	Root nodules	nd
Rosaceae	*Rubus*	*(Actinobacteria)*	Root nodules	nd
Pteridophytes	*Azolla*	*Anabaena*	Heterocysts in cavities of dorsal leaf lobes	40–120
Cycads	*Ceratozamia*	*Nostoc*	Coralloid roots	19–60
Lichens	*Collema*	*Nostoc*	Interspersed between fungal hyphae	nd

Source: Kwon & Beevers (1992), Gault et al. (1995), Peoples et al. (1995), Vance (2002).
* Only a limited number of species are listed, just to provide an example; nd is not determined.

capable of fixing atmospheric N_2. It shows that N_2-fixing organisms can be very significant for the input of N into natural and agricultural systems.

3.2 Host–Guest Specificity in the Legume–Rhizobium Symbiosis

The associations between legumes and rhizobia have been studied most elaborately. Many are highly specific. For example, *Rhizobium meliloti* will infect *Medicago truncatula* (medic), *Melilotus alba* (honey clover), and *Trigonella coerulea* (fenugreek), but not *Trifolium repens* (white clover) or *Glycine max* (soybean). *Bradyrhizobium japonicum* will nodulate *Glycine max* (soybean), but not *Pisum* sativum (pea) and *Medicago truncatula* (medic). Other rhizobia (e.g., *Rhizobium* strain NGR234) may infect up to 100 host species, from different genera, including *Parasponia andersonii*, a nonlegume, but this is exceptional. What determines the specificity and why does this specificity vary among different rhizobia? To answer these questions, we first discuss the infection process in more detail.

3.3 The Infection Process in the Legume–Rhizobium Association

Nodule formation in legumes such as *Pisum sativum* (pea) and *Glycine max* (soybean) is preceded by the release of **specific phenolic compounds** (**flavonoids**: flavones, flavanones, or isoflavones) and betaines from the legume roots (Phillips et al. 1994). The same or similar flavonoids are induced as antibiotics (phytoalexins) upon infection by pathogenic microorganisms (Sect. 3 of Chapter 9C on effects of microbial pathogens). Subtle differences between host plant species, of which we are only just beginning to understand the details, determine if an interaction between a bacterium and a plant results in symbiosis or pathogenesis. The flavonoids bind with a bacterial gene product, and then interact with a specific promoter in the genome of rhizobium. This promoter is associated with the genes responsible for inducing nodulation (the nodulation, or ***nod* genes**). As detailed in the following sections, the products of these genes, the **Nod factors**, induce **root-hair curling** on the plant and **cortical cell divisions**, which are among the earliest, microscopically observable events in the nodulation of most legume species. Nod factors are not required for symbiosis in some legumes, e.g., *Aeschynomene* species forming a symbiosis with photosynthetic *Bradyrhizobium* species (Giraud et al. 2007). Rhizobia may also enter through cracks in the epidermis, associated with lateral-root formation, or wounds; for 25% of all legumes this is the only way of entry (Sprent 2007).

The actinorhizal symbiosis between plant species like *Alnus glutinosa* (black alder) and the Actinobacteria *Frankia* also involves the release of specific compounds (flavonols) that enhance the level of

nodulation, but their exact role in the process is unknown (Van Ghelue et al. 1997, Vessey et al. 2005). Very little is known about the chemical nature of attractants from hosts to Cyanobacteria (Vessey et al. 2005).

3.3.1 The Role of Flavonoids

To some extent **specificity** between the host and rhizobium is determined by the type of **flavonoids** released by the host and by the sensitivity of the rhizobium promoter for a given type of flavonoid (Table 5). Rhizobium species that form symbioses with a broad range of plant species respond to a wider range of flavonoids than those that are more specific, but, if the flavonoid concentration is increased, a response may occur, even in those more specific rhizobia. In addition, nonlegumes may also exude flavonoids, and several legumes exude flavonoids that also activate the promoter of rhizobium species that are unable to establish a symbiosis. Other factors must, therefore, also contribute to specificity (Spaink 1995). What ultimate effects do the flavonoids have in rhizobium?

To study the effect of flavonoids on rhizobium an appropriate assay is required that is less elaborate than measuring root-hair curling (Maxwell et al. 1989). A construct has been made, coupling the gene from *Escherichia coli* that encodes β-galactosidase to the promoter of the *nod* genes. The activity of the enzyme β-galactosidase is then measured in a simple spectrophotometric assay. In this way, the relative effect of various flavonoids can be assessed by determining the activity of β-galactosidase, rather than the extent of root-hair curling. In the following section we examine the kind of products produced by the bacterial *nod* genes.

3.3.2 Rhizobial *nod* Genes

There are three types of rhizobial *nod* genes (Fig. 15). First, all rhizobia have a *nod* gene that is transcribed constitutively and probably confers some host specificity. The product of this gene binds with flavonoids produced by the host plant to activate the common *nod* genes, which are found in all rhizobium species and lead to the production of a bacterial **lipochitooligosaccharide**. The flavonoids also activate the transcription of host-specific *nod* genes, which encode enzymes that "decorate" this lipochitooligosaccharide. The "decorated" lipochitooligosaccharides are known as the **Nod factors**. The lipid component of the Nod factor allows penetration through membranes. Different side groups are added to the backbone of this molecule, and this confers the specificity of a certain rhizobium (Fig. 15). Rhizobial species with a broad specificity produce many different Nod factors, as opposed to ones with a narrow host range. That is, the structure of the lipochitooligosaccharide determines if it will be recognized as a symbiont or as a pathogen by a potential host plant. Because the Nod factor is effective at concentrations as low as 10^{-12} M, a plant **receptor** must be involved, and evidence for this has recently been obtained (Radutoiu et al. 2003, Oldroyd et al. 2005). Rhizobial Nod factors trigger *nod* gene transcription in plant epidermal cells within 1 hour of exposure;

Table 5. Comparisons of indeterminate and determinate nodules.

Parameter	Indeterminate	Determinate
Nodule initiation	Inner cortex	Outer cortex
Cell infection through	Infection threads	Infection threads and cell division
Meristem	Persistent (months)	Nonpersistent (days)
Bacteroid size	Larger than bacteria	Variable, although usually not too much larger than bacteria
Peribacteroid membrane	One bacteroid per symbiosome	Several bacteroids per symbiosome
N_2 fixation products transported	Amides usually	Ureides usually
Infected cells	Vacuolate	Nonvacuolate
Geographical origin	Temperate	Tropical to subtropical
Genera	*Medicago, Trifolium, Pisum, Lupinus*	Isoflavones
nod Gene inducers	Flavones, isoflavones	*Glycine, Phaseolus, Vigna*
Ploidy level of bacteroids	Polyploid	Diploid
Viability of bacteroids	Nonviable upon release in soil	Viable upon release in soil

Source: Vance (2002), Vessey et al. (2005), Mergaert et al. (2006).

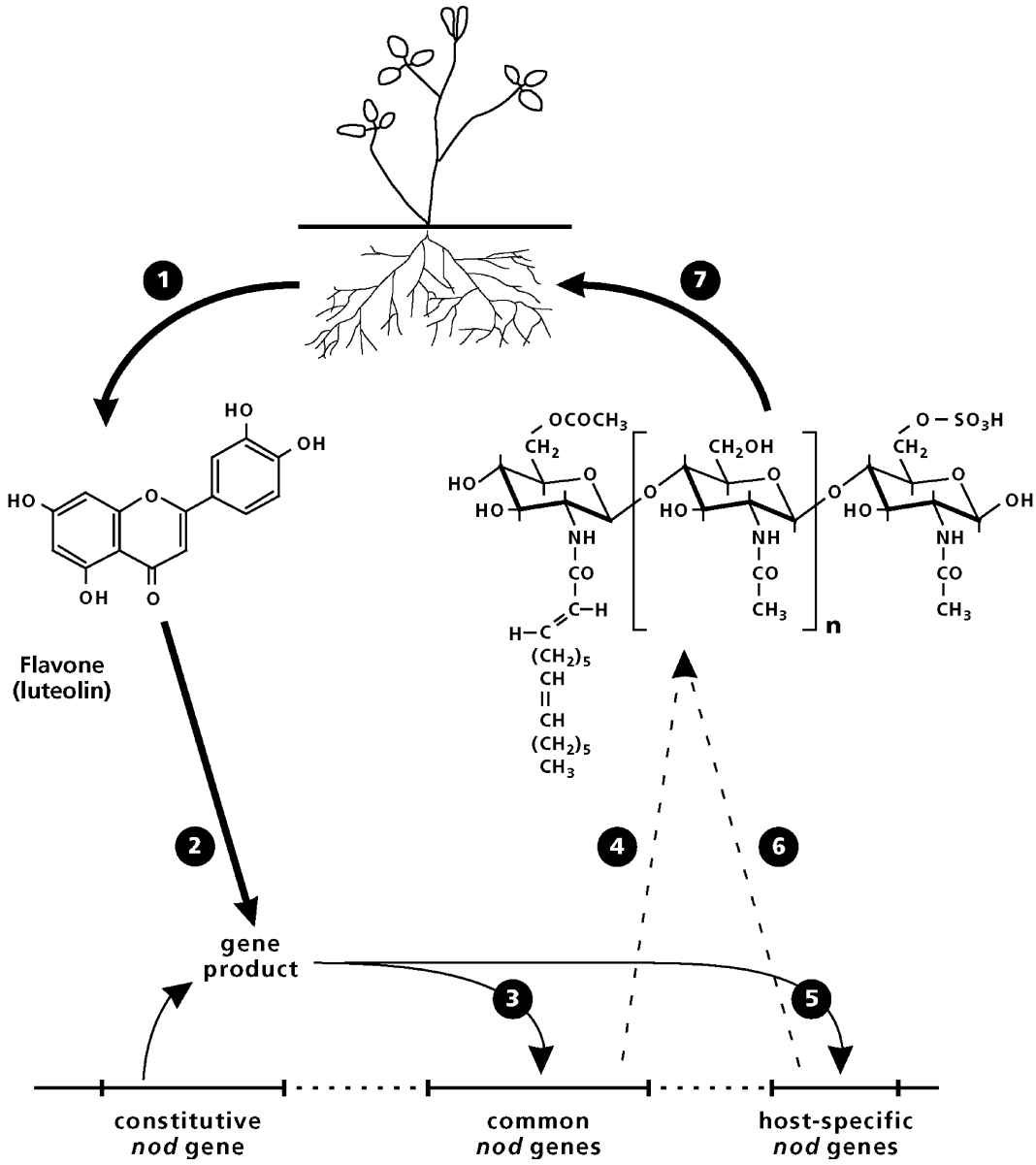


FIGURE 15. Symbiotic signaling between legume plants and rhizobia. (1) Flavonoids are exuded by the legume roots. (2) The flavonoids bind to the gene product of a constitutively expressed nodulation (*nod*) gene. (3) After this binding, common *nod* genes are activated. (4) This leads to the production of a lipochitooligosaccharide. (5) The flavonoids also activate the transcription of specific *nod* genes. (6) The products of the specific *nod* genes lead to modification of the lipochitooligosaccharides and the formation of nodulation (nod) factors, which confer specificity. (7) The Nod factors are recognized by receptors on the surface of the legume-host's roots.

maximum expression is in the differentiating region of the root between the growing root tip and the zone of root-hair emergence, where the nodulation process is initiated. An increase of the cytosolic Ca concentration, which originates from intracellular sources and from the apoplast, appears to play a role in the signal-transduction pathway that starts with the perception of the Nod factor and leads to *nod* gene expression (Pingret et al. 1998).

Much less is known about plant factors determining if a rhizobium will recognize a plant as an appropriate host. Flavonoids offer only a partial explanation. When rhizobial genes that confer host specificity are transferred to a rhizobium strain with a different specificity, these genes alter the bacterial acidic polysaccharide structure and in situ binding to the host's root hairs. Furthermore, introducing the genes encoding specific root-hair proteins (**lectins**) into *Trifolium repens* (white clover) allows nodulation of clover roots by a *Rhizobium* strain, which is usually specific for *Pisum sativum* (pea). It has therefore been thought that the host–*Rhizobium* specificity involves the interaction of the root hair lectins with specific carbohydrates on the bacterial surface (Diaz et al. 1989).

3.3.3 Entry of the Bacteria

After the release of flavonoids by the host and the release of the Nod factors by rhizobium, the bacteria multiply rapidly in the rhizosphere. The bacteria may adhere to root hairs and affect those that have just stopped growing. Younger and older root hairs are not affected. Alternatively, entry may occur through cracks in the epidermis or wounds. This is the only way of entry for 25% of all legumes (Sprent 2007) as well as for entry into nonlegumes, e.g., *Oryza sativa* (rice), *Triticum aestivum* (wheat), and *Zea mays* (corn) (Webster et al. 1997). However, upon entry into nonlegumes, with the exception of *Parasponia* species, no nodules are formed.

When rhizobia adhere to root hairs, the cell wall of the affected root hair is then partly hydrolyzed at the tip. In this process the root hairs curl, attaching the bacteria to the root hair. On those locations on the root hairs that have become deformed due to the presence of rhizobia, the cell wall is degraded, allowing the bacteria to enter. An **infection thread** is formed by invagination of the cell wall. This thread consists of cell-wall components similar to those that form the normal root-hair cell wall. The infection thread grows down the root hair at a rate of 7–10 $\mu m\ h^{-1}$ and provides a conduit for bacteria to reach the root cortex. The tip of the transcellular infection thread appears to be open; sealing of the thread tip results in abortion of the infection thread. The formation of the infection thread may well be analogous to the enlargement of epidermal cell walls, in response to a pathogen's attempted penetration (Vance 2002).

Only 1–5% of all root hairs become infected, and only 20% of these infections result in nodules. Why are most of the infections unsuccessful? This is likely to be due to the production of **chitinases** by the plant. These enzymes hydrolyze the lipochitooligosaccharide Nod factor (Mellor & Collinge 1995). Legumes contain different chitinases. In an early stage of infection the host produces a chitinase that breaks down the Nod factors of *Rhizobium* species that are not suitable guests. In this way they prevent the entry of bacteria that cannot form a symbiosis. Chitinases are therefore another factor that confers **host–guest specificity**. At a later stage, different chitinases are produced that are effective against the Nod factor of "homologous" rhizobium bacteria, i.e., suitable guests. This is probably a mechanism to control the entry of rhizobium and to prevent more nodules being formed than can be supported by the host. In addition, the breakdown of Nod factors prevents entering bacteria from being erroneously recognized as pathogens. *Rhizobium* strains that overproduce the Nod factor do indeed lead to a defense response. Not only is the Nod factor broken down by plant chitinase activity, but expression of the bacterial *nod* genes is also suppressed at a later stage of infection. Plant phenolics may play a role in this suppression. If the *Rhizobium* fails to recognize the plant-derived suppressor molecule, the bacteria may be recognized as a pathogen and further development stops. This offers another possibility for **host–guest specificity**. The train of events that likely plays a role in the early stages of infection and the role of chitinases is outlined in Fig. 16.

If the infection is successful, then specific genes are activated in the cortex and pericycle which allows the formation of an infection thread through which the bacteria enter (Vessey et al. 2005). Cell divisions start in the inner cortex (indeterminate nodules) or outer cortex (determinate nodules), opposite protoxylem poles, so that a new **meristem** is formed due to the presence of the rhizobia. This meristem gives rise to the **root nodule**. The infection thread grows inward, and, finally, the bacteria are taken up into the cytoplasm of the parenchyma cells of the center of the developing nodule. Inside the infected host plant cell, the bacteria continue to divide for some time, now differentiating into **bacteroids**, which have a diminished ability to grow on laboratory culture media; they may be greatly enlarged with various shapes. In most legumes, bacteroids are enclosed within a **peribacteroid membrane**, to form a **symbiosome** (White et al. 2007). Most symbiosomes are of a similar volume, but in some nodules, each symbiosome contains a single, enlarged pleomorphic bacteroid, whereas there may be several (up to 20) smaller, rod-shaped bacteroids in others. Symbiosomes with a single bacteroid are more typical of elongate, cylindrical nodules of the so-called **indeterminate** class, as

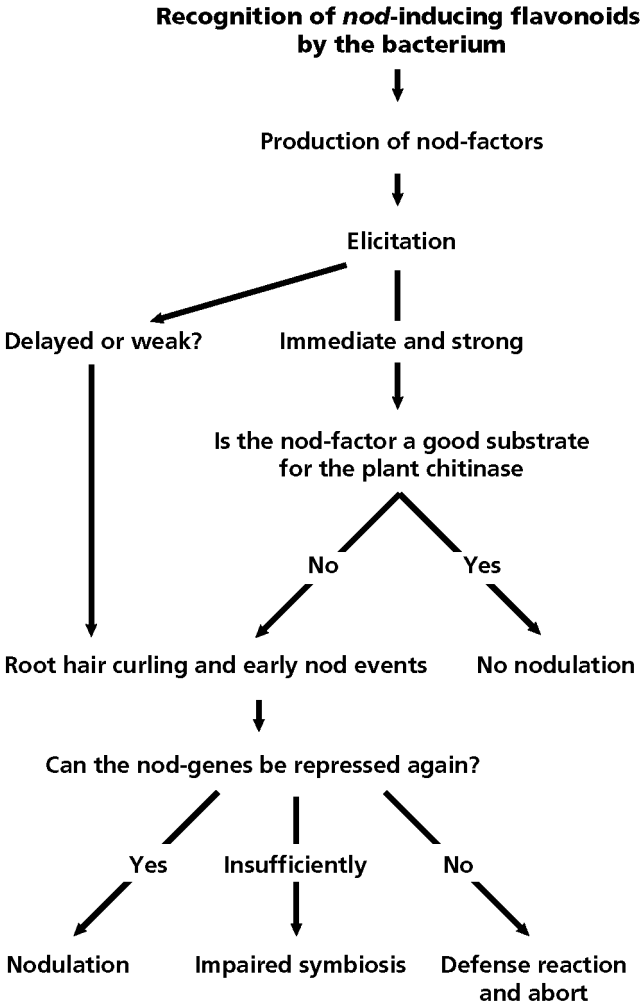


FIGURE 16. Tentative scheme to account for events that determine the establishment of a functional symbiosis (effective nodulation) between rhizobium and a host legume. "Elicitation" is a combination of the elicitor activity of a specific bacterial Nod factor and the sensitivity of the plant to that elicitor (Mellor & Collinge 1995). Reproduced with kind permission of Oxford University Press.

found on *Trifolium repens* (white clover) or *Pisum sativum* (pea) (Table 5). These nodules have a persistent meristem. Bacteroids in indeterminate nodules are polyploid, and have lost their ability to divide; hence they are nonviable when released from nodules (Mergaert et al. 2006). Symbiosomes with several bacteroids are common in spherical, **determinate** nodules (with no meristem), such as those of *Vigna unguiculata* (cowpea) or *Glycine max* (soybean). Bacteroids in determinate nodules are diploid, like free-living rhizobia, and can divide in soil upon release from nodules. The ploidy level of the bacteroids is controlled by the host (Mergaert et al. 2006). A few legumes have nodules in which there are no symbiosomes, the bacteria being retained within multiply branched infection threads. Mature nodules of the determinate and indeterminate types are strikingly different, but their initiation is rather similar (Sprent 2007).

3.3.4 Final Stages of the Establishment of the Symbiosis

Each **infected cell** may contain many hundreds of symbiosomes. The symbiosome membrane (**peribacteroid membrane**) originates from an invagination and endocytosis of the plasma membrane of the infected cortical cells. This membrane acts as a selective permeability barrier to metabolite exchange between the bacteroids and the cytosol of the infected cells. Interspersed between the infected cells of many nodules are smaller, **uninfected cells**, which occupy about 20% of the total volume of the central zone of the nodules of *Glycine max* (soybean). **Plasmodesmata** connect uninfected with infected cells and with other uninfected cells in the central zone of the nodule. These plasmodesmata allow for the massive transport of carbon from the uninfected cells to the infected ones and of

nitrogenous compounds in the reverse direction (Brown et al. 1995). Both infected and uninfected cells contain numerous plastids and mitochondria. Uninfected and infected cells in nodules have different metabolic roles in symbiotic N_2 fixation (Day & Copeland 1991). The central tissue of some nodules, however, contains no uninfected cells.

In *Glycine max* (soybean) nodules, an outer layer of cortical cells surrounds an endodermal cell layer, which in turn encloses several layers of subcortical cells. The central zone of the nodules contains several thousand infected cells. The nodule is connected with the **vascular tissue** in the stele, due to the proliferation of cells from the pericycle.

The pattern of gene expression in the host cells that are part of the nodules is altered by the presence of the bacteria, resulting in the synthesis of many different proteins, known as **nodulins**. Some of these nodulins have been characterized biochemically, including the O_2 carrier **leghemoglobin** and nodule-specific forms of the enzymes uricase, glutamine synthetase, and sucrose synthase.

3.4 Nitrogenase Activity and Synthesis of Organic Nitrogen

Biological reduction of N_2 to NH_3 is catalyzed by **nitrogenase**, in a highly **O_2-sensitive process**. This O_2 sensitivity accounts for the pink color of the nodule tissue which is due to the presence of **leghemoglobin** in the cytoplasm of the infected legume cells or **hemoglobin** in nodules of species living symbiotically with *Frankia* (Gualtieri & Bisseling 2000). This heme-protein may comprise 35% of the total nodule soluble protein. Leghemoglobin is related to the myoglobin of mammalian muscle; its protein component is encoded by the DNA of the plant. The enzymes responsible for the synthesis of the O_2-binding heme-group of the protein in legume nodules are encoded both in rhizobium and in the host [e.g., *Pisum sativum* (pea)]. Because the enzymes are up-regulated in the infected host cells in root nodules that synthesize the other component of leghemoglobin, the heme moiety is probably synthesized by the macrosymbiont (Santana et al. 1998). Leghemoglobin plays a role in the **O_2 supply** to the bacteroid. It has a high affinity for O_2 and a relatively fast O_2-dissociation rate, which ensures sufficiently rapid **O_2 supply** for the highly active respiratory processes in the plant and bacteroid compartment while maintaining a low concentration of free O_2 (between 3 and 30 nM). The latter is very important because **nitrogenase**, which is the enzyme responsible for the fixation of atmospheric N_2 to NH_3, is rapidly damaged by **free O_2** (Arredondo-Peter et al. 1998, Gualtieri & Bisseling 2000).

To control the O_2 supply and O_2 concentration to and within infected cells, **nodule permeability to O_2 diffusion** varies within seconds to hours in response to changes in carbohydrate supply via the phloem, adenylate demand, and O_2 status. This permeability control is associated with the reversible flow of water into or out of intercellular spaces. When nodulated *Glycine max* (soybean) plants are exposed to treatments that decrease the nodules' O_2 permeability, the K^+ concentration in the nodule cortex increases relative to that in the central zone of the nodules. On the other hand, treatments that increase O_2 permeability have the opposite effect. The energy-dependent coupled movement of ions and water into and out of infected cells offers a possible mechanism for diffusion barrier control in legume nodules (Wei & Layzell 2006).

The bacteroids contain **nitrogenase**, which is an enzyme complex that consists of two proteins. One, nitrogenase-reductase, is an Fe–S-protein that accepts electrons, via an intermediate electron carrier, from NADPH and then binds ATP. At the same time, the other subunit (an Fe–Mo-protein) binds N_2. Reduction of this N_2 occurs if the two subunits have formed an active complex. A minimum of 12, and possibly as many as 16 ATP are required per N_2; therefore the overall equation is:

$$\begin{aligned} N_2 + 8\ H^+ + 8\ e^- + 16\ ATP \\ \rightarrow 2\ NH_3 + H_2 + 16ADP + 16\ P_i \end{aligned} \quad (1)$$

Most of the N_2 fixed by the bacteroids is released as NH_3 to the peribacteroid space and then as NH_4^+, via a voltage-driven channel across the peribacteroid membrane, to the cytosol of the nodule cells (Fig. 17; Mouritzen & Rosendahl 1997). Alternatively, NH_3 may be converted into alanine, and then exported (Fig. 17; White et al. 2007). A nodule-specific glutamine synthetase is expressed in the cytosol of infected cells. Glutamine 2-oxoglutarate aminotransferase (GOGAT) then catalyses the formation of two molecules of glutamate from one molecule of glutamine. The major N-containing products exported via the xylem are the **amides** asparagine and glutamine in such species as *Pisum sativum* (pea), *Medicago sativa* (alfalfa), and *Trifolium repens* (white clover). These products are typical for nodules that are elongate-cylindrical with **indeterminate** apical meristematic activity (Fig. 18, Table 5). In *Phaseolus vulgaris* (common bean) and *Glycine max* (soybean), the products are predominantly **ureides**: allantoin and allantoic acid (Fig. 18, Table 5). Nodules exporting these compounds are spherical

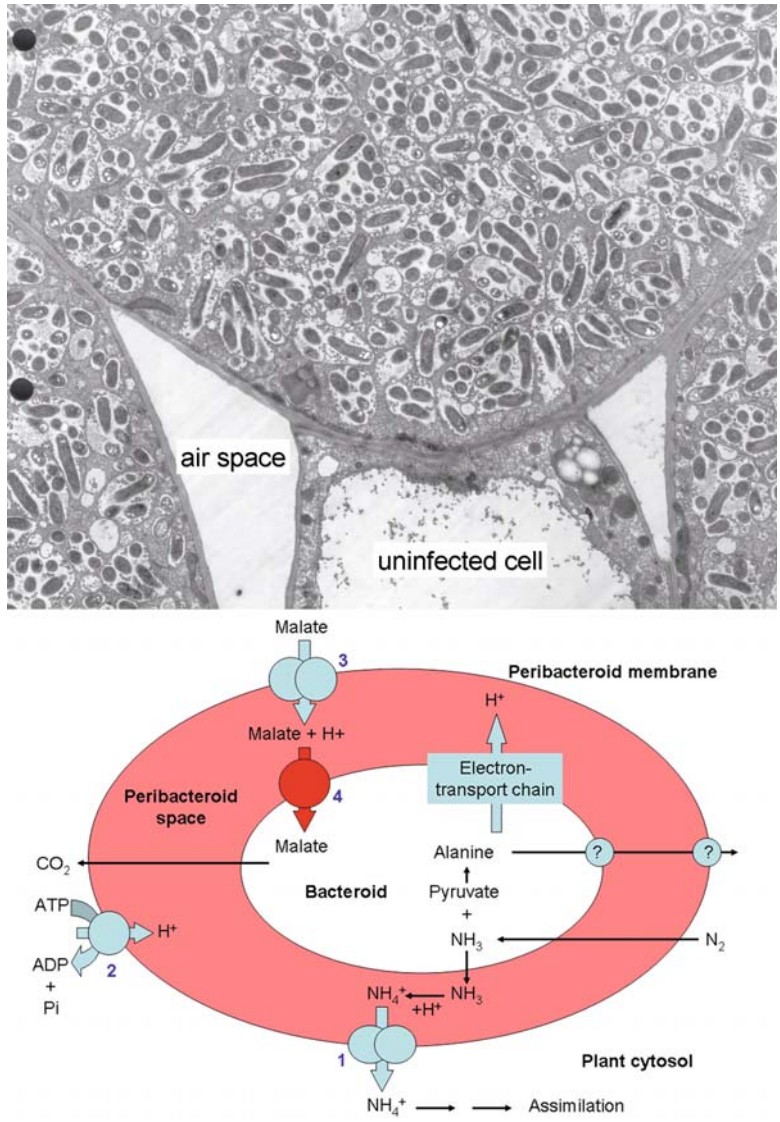


FIGURE 17. (Top) Electron micrograph of a nodule of *Glycine max* (soybean), infected with *Bradyrhizobium japonicum*. Three infected cells and one uninfected cell are shown, with two air spaces in between. Note that the uninfected cell is much smaller than the infected ones, and that the bacteroids are grouped as "symbiosomes", surrounded by a peribacteroid membrane (courtesy D. Price, Research School of Biological Sciences, Australian National University, Canberra, Australia). (Bottom) Scheme of N_2-fixation and NH_3 production in bacteroids. NH_3, being an uncharged molecule, can cross the bacteroid membrane and arrives in the peribacteroid space, where it picks up a proton and becomes NH_4^+. Subsequently, NH_4^+ leaves the symbiosome via a monovalent cation channel (1). Alternatively, NH_3 may react with pyruvate, producing alanine. An ATPase in the peribacteroid membrane (2) creates a membrane potential (positive inside of the symbiosome), which drives the passive uptake of negatively charged organic acids (predominantly malate and succinate) into the symbiosome (3). Alternatively, organic acids may arrive symplastically from neighboring uninfected cells. An electron-transport chain in the bacteroid membrane (etc.) creates a proton-motive force, which drives the uptake of malate (and succinate) into the bacteroids via a H^+-co-transport mechanism (4) [Whitehead et al. (1995), White et al. (2007)].

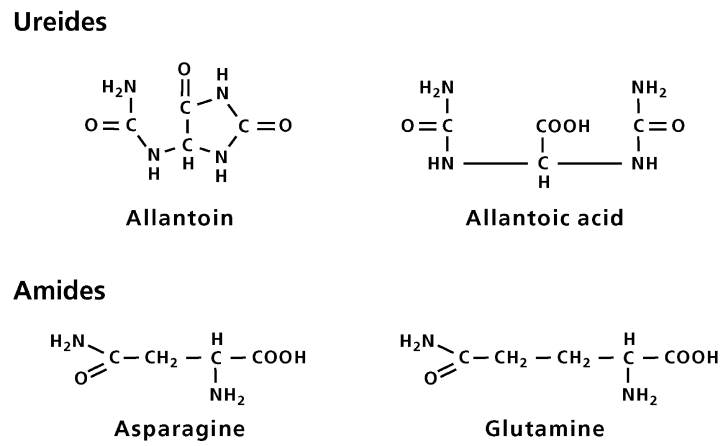


FIGURE 18. Major nitrogen transport products from legume nodules. The C:N ratio of ureides is 1:1, whereas that of amides is 2:1 (asparagine) or 2.5:1 (glutamine).

with **determinate** internal meristematic activity (Vessey et al. 2005).

The ureides released to the xylem of plants with **determinate** nodules are products that are only found when the symbiotic plants are fixing N_2 (Peoples et al. 1996). Hence the concentration of these compounds in the xylem sap, relative to the total amount of N transported in the xylem, has been used to estimate the proportion of N derived from N_2 fixation, as opposed to the assimilation of combined N (Peoples et al. 1996). The amides released to the xylem of plants with **indeterminate** nodules are also found when these plants grow nonsymbiotically. In fact, they are not even typical for legumes. Hence, they cannot be used as "markers" for symbiotic N_2 fixation.

3.5 Carbon and Energy Metabolism of the Nodules

Carbohydrates are supplied via the phloem to the nodules, where they are rapidly converted in the plant compartment to **dicarboxylic acids** (malate, succinate), predominantly in the uninfected cells in the nodules (Lodwig & Poole 2003, White et al. 2007). Malate and succinate are the major substrates for the bacteroids (Fig. 17). How do the infected cells prevent a large part of the organic acids from being oxidized via the nonphosphorylating alternative path in a situation where the demand for organic acids of the bacteroids is very large (Sect. 2.3 of Chapter 2B on plant respiration)? Mitochondria from nodules have very little alternative path capacity; the little capacity they have is restricted to the uninfected cortical cells, rather than to the infected ones (Table 6). There is, therefore, no risk of oxidizing the organic acids destined for the bacteroids.

Apart from N_2, H^+ is also reduced by nitrogenase [see Equation (1) in Sect. 3.4], leading to the production of H_2. Most rhizobia, however, contain **hydrogenase**, which is an enzyme that consumes H_2, using it as an electron donor. The characteristic of nitrogenase to reduce acetylene (ethyne) to ethylene (ethene) is frequently used to assay nitrogenase activity in vivo. Because the assay itself,

TABLE 6. Cyanide-resistant, SHAM-sensitive respiration in infected and uninfected cells isolated from *Glycine max* (soybean).*

	O_2 consumption [nmol mg^{-1} (protein) min^{-1}]		
Cells from root nodules	Control	KCN-resistant respiration	KCN resistant respiration (%)
Infected	60	0	0
Uninfected	45	22	49

Source: Kearns et al. (1992).
* Measurements were made on isolated mitochondria from different tissues, as well as on infected and uninfected cells from root nodules.

however, interferes with the process of N_2 fixation, it should only be considered as a *qualitative* to *semi-quantitative* indicator for the occurrence of nitrogenase activity, rather than as a good *quantitative* measure for its actual activity (Hunt & Layzell 1993, Vessey 1994).

3.6 Quantification of N_2 Fixation In Situ

The contribution of symbiotic N_2 fixation to the total accumulation of N in the above-ground biomass of a crop or a plant community can be determined by applying ^{15}N-labeled inorganic N ($^{15}NO_3^-$ or $^{15}NH_4^+$) separately to N_2-fixing plants and reference plants (i.e., nonfixing species or mutants). For example, it can be given to a plant community that consists of both N_2-fixing species (e.g., clover) and other species (e.g., grasses) (^{15}N is a nonradioactive isotope of N). The grasses have a $^{15}N/^{14}N$ ratio, which is used as a reference. N_2 fixation in the N_2-fixing clovers will "dilute" their ^{15}N concentration. The extent of the dilution is used to calculate the contribution of fixation to the total amount of N that accumulates in the clover plants. The contribution of N_2 fixation to the total amount of N that accumulates in the plant may amount to 75 and 86% in *Trifolium repens* (white clover) and *Trifolium pratense* (strawberry clover). Transfer of N is most likely via release of ammonium and amino acids from legume roots (Paynel et al. 2001), but under some conditions N transfer is not detected (McNeill & Wood 1990). The contribution depends on the amount of inorganic combined N that the plants receive from soil and fertilizer, and also varies with the developmental stage of the plant and the time of the year (Fig. 19). The overwhelming importance of symbiotic N_2 fixation in some agricultural systems is illustrated in Table 7.

Sometimes the **natural abundance** of ^{15}N in the soil is used to quantify N_2 fixation (Table 2 in Sect. 2.4). Instead of adding ^{15}N-labeled inorganic combined N, the natural abundance of N in the soil can be used. The natural abundance of soil N is likely to differ from that of N_2 in the atmosphere, due to discrimination against the heavy isotope in various biological processes (e.g., nitrification and denitrification). N in the soil is therefore "enriched" with the heavy isotope, relative to N_2 in the air. To apply this technique in situ, reliable control plants have to be used. As discussed in Sect. 2 (Table 2), this is not always easy, if it is at all possible (Handley et al. 1993).

The ^{15}N technique referred to earlier has also been used to demonstrate a significant transfer of

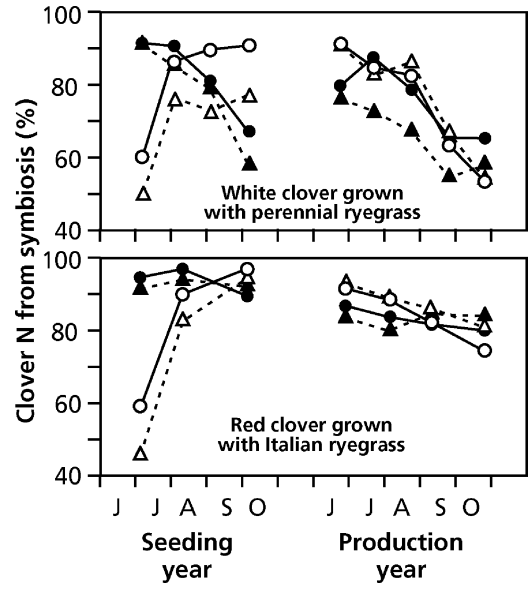


Figure 19. The contribution of symbiotic nitrogen fixation to the total N accumulation in the above-ground biomass of *Trifolium repens* (white clover) (*top*) and *Trifolium pratense* (strawberry clover) (*bottom*). The white clover plants grew in a mixed culture with *Lolium perenne* (perennial ryegrass) and the strawberry clover with *Lolium multiflorum* (Italian ryegrass). Circles: no N fertilizer; triangles: 30 kg N ha^{-1} per cut; open and closed symbols refer to different years (Boller & Nösberger 1987).

Table 7. Symbiotic N_2 fixation by some legume crops[1] and native species in their natural environment in Brazil[2].

Species	N_2 fixed kg ha^{-1} per season	Plant N absorbed from atmosphere (%)
Medicago sativa	440–780	65–96
Glycine max	120	53
Lotus corniculatus	92	55
Lupinus angustifolius	170	65
Medicago sativa	180	70
Phaseolus vulgaris	65	40
Pisum sativum	72	35
Trifolium pratense	170	59
Vicia faba	151	nd
Vigna angularis	80	70
Chamaecrista species	nd	66–79
Mimosoid legumes	nd	42–63
Papilionoid legumes	nd	68–79

Source: [1]Gault et al. (1995), Vance (2002); [2]Sprent et al. (1996).

N from symbiotic plants to neighboring grasses (up to 52 kg N ha^{-1} $year^{-1}$; on average a value of 17 kg ha^{-1} $year^{-1}$ is found). Conditions favoring N_2 fixation by the legume, such as a high irradiance, a favorable temperature, long days, and a relatively high P_i supply, enhance the transfer of N from the legume to the nonfixing neighbors. This transfer is to some extent the result of the uptake of nitrogenous compounds released after decomposition of parts of the legumes. Some of it is also due, however, to the exudation of nitrogenous compounds by the legumes, followed by absorption by the nonfixing plants. Some transfer of N may occur through mycorrhizal hyphae (Sect. 2.3.1).

The maximum yield of *Lolium rigidum* (annual ryegrass) is reached at a much lower supply of P_i than that of *Trifolium subterraneum* (subclover), at least when both species are grown in monoculture (Bolan et al. 1987). This demand for a higher P is fairly common for nodulated legumes, although it is by no means universal (Koide et al. 1988, Sprent 1999). The high demand for P of many crop legumes may reflect their adaptation to soils with a high availability of P_i, and it points out that a benefit from such legumes can only be expected when these have access to sufficient P_i. Apart from P_i, also Mo and S have to be available to the legume to allow symbiotic N_2 fixation.

What is the role of N_2-fixing species in more biodiverse grasslands? To address this question, test plants ("phytometers") can be planted in plots under investigation, to sample their above-ground biomass at a later stage (both N concentration and natural abundance of ^{15}N: $\delta^{15}N$). Phytometers in a recent study belonged to four "**plant functional groups**" (Chapter 9E): *Festuca pratensis* (meadow fescue), *Plantago lanceolata* (snake plantain), *Knautia arvensis* (field scabious), and *Trifolium pratense* (strawberry clover). Significantly lower $\delta^{15}N$ values and higher N concentrations and N contents were found in all phytometer species growing with legumes, indicating a facilitative role for legumes in these natural grassland ecosystems. The magnitude of the positive interactions depends on the exact phytometer species, but increased N uptake in communities containing legumes is found in all three nonlegume phytometer species, with a subsequent strong increase in biomass in the grass *Festuca pratensis* across all diversity levels, and a lesser biomass gain in *Plantago lanceolata* and *Knautia arvensis*. In contrast, the legume phytometer species *Trifolium pratense* is negatively affected when other legumes are present in their host communities across all diversity levels (Temperton et al. 2007).

3.7 Ecological Aspects of the Nonsymbiotic Association with N_2-Fixing Microorganisms

Next to the truly symbiotic associations that lead to N_2 fixation, as discussed in Sect. 3.3, a somewhat looser association between *Azospirillum* and higher plants (especially grasses) has been investigated. Inoculation of the soil in which *Zea mays* (corn) plants are grown with *Azospirillum* bacteria significantly enhances the yield of the corn plants, especially when the N supply is relatively low (Table 8). It is by no means certain, however, that this is a direct result of the fixation of N_2 by the *Azospirillum* bacteria. These organisms are more likely to enhance the growth of higher plants in a different manner, such as the production of phytohormones (Dobbelaere et al. 1999). In a comparison of three cultivars of *Triticum aestivum* (wheat), grown with *Azospirillum brasiliense*, most N_2 is fixed in the rhizosphere of Al-resistant cultivars. Because these cultivars also exude more dicarboxylic acids (Sect. 3.1 of Chapter 6 on mineral nutrition), it has been suggested that fixation is enhanced by the excretion of

Table 8. The effect of inoculation with *Azospirillum brasiliense* on the production of *Zea mays* (corn) plants as dependent on the N supply.*

N supply ($g\ L^{-1}$)	Shoot dry mass (g)		Root dry mass (g)		Relative increment of total plant mass (%)
	Inoculated	Control	Inoculated	Control	
0	0.49	0.32	0.36	0.27	44
0.04	0.97	0.66	0.76	0.53	45
0.08	1.84	1.23	0.97	0.86	34
0.16	2.93	2.52	1.96	1.70	16

***Source*: Cohen et al. (1980).**
* **N was supplied as NH_4NO_3.**

these organic molecules (Christiansen-Weniger et al. 1992). Microorganisms might promote plant growth in many other ways, such as **suppression of pathogenic organisms** and the **production of vitamins**.

In some areas in Brazil, *Saccharum officinarum* (**sugarcane**) has been grown continuously for more than a century without any nitrogenous fertilizer. Although it had long been suspected that substantial N_2 fixation occurs in such systems, none of the N_2-fixing bacteria isolated from the rhizosphere of *Saccharum officinarum* occur in large enough numbers to account for the high rates of N_2 fixation found in these crops. An acid-tolerant N_2-fixing bacterium (*Glucoacetobacter diazotrophicus* and a range of others) has been identified in the **intercellular spaces** of sugarcane stem parenchyma (Dong et al. 1994, James et al. 1994). These spaces are filled with a solution that contains 12% sucrose (pH 5.5). *Glucoacetobacter diazotrophicus* has most unusual growth requirements; it shows optimal growth with 10% sucrose and pH 5.5. It will grow in a medium with 10% sucrose and rapidly acidifies its surroundings by the formation of acetic acid. It has been isolated from sugarcane tissues, but was not found in the soil between rows of sugarcane or in grasses from the same location. The apoplastic fluid occupies approximately 3% of the stem volume, which is equivalent to 3 tons of fluid per hectare of the sugarcane crop. It has been suggested that this amount suffices to make the sugarcane independent of N fertilizers.

Enterobacter agglomerans, *Herbaspirillum seropedicae*, and *Klebsiella terrigena* are also believed to be able to fix atmospheric N_2 in the apoplast of plants that have high apoplastic sugar concentrations. Some of these (e.g., *Herbaspirillum seropedicae*) are pathogens on certain grass species which restricts their potential use as inoculants in agriculture (Palus et al. 1996, Triplett 1996). To date, with the exception of sugarcane, little success has been attained in elucidating which endophyte is really responsible for the observed biological N_2 fixation, and in what site, or sites, within plants the N_2 fixation mainly occurs. Until such important questions are answered, further developments or extension of this novel N_2-fixing system to other economically important nonlegumes (e.g., cereals) will be seriously hindered (Boddey et al. 2003).

3.8 Carbon Costs of the Legume–Rhizobium Symbiosis

Because all the organic acids required for symbiotic N_2 fixation by rhizobium and for maintenance of the root nodules come from the plant (Fig. 17), there are costs involved in this symbiotic system for the higher plant. These costs exceed those required for assimilation of NO_3^- or NH_4^+. They have been estimated for an association of *Trifolium repens* (white clover) and rhizobium, in which the clover plants are totally dependent on the microsymbiont for their supply of N (Fig. 20). In this symbiotic system, the N_2 fixation is briefly interrupted by decreasing the O_2 concentration that surrounds the plants, but kept sufficiently high to fully maintain aerobic

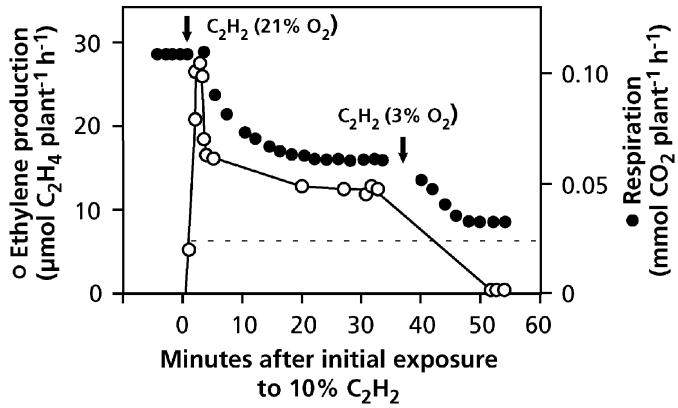


FIGURE 20. Respiration and acetylene reduction (measured as ethylene production) in roots of *Trifolium repens* (white clover) at either high or low O_2 concentration. The low O_2 concentration was sufficiently high to maintain aerobic metabolism of the plant cells, but virtually completely abolished the activity of the N_2-fixing activity of the bacteroids. The decline in respiration after adding acetylene reflects the sensitivity of the nodules to "manipulations". It also shows that this technique cannot be used to give reliable quantitative estimates of the rate of N_2 fixation (Ryle et al. 1985). Reproduced with kind permission of Oxford University Press.

metabolism of the plant. It is sufficiently low, however, to completely block the respiration and N_2 fixation by the bacteroids. By relating the decrease in respiration upon blocking the N_2 fixation to the activity of N_2 fixation as determined from the accumulation in the clover plants, **carbon costs** per unit fixed N are calculated. The costs for N_2 fixation amount to approximately 25% of all the carbon fixed in photosynthesis per day. This proportion is rather high when compared with the figures for N acquisition by nonsymbiotic plants given in Table 2 of Chapter 2B on respiration: 4–13% when plants are grown at an optimum nutrient supply. At a limiting nutrient supply, the percentage is likely to be more similar to that of the costs of N_2 fixation.

Because of the high carbon costs of symbiotic N_2 fixation, it has been suggested that **elevated atmospheric CO_2 concentrations** will stimulate this process. However, a meta-analysis shows that symbiotic N_2 fixation is stimulated by elevated atmospheric [CO_2] only when sufficient soil nutrients, other than N, are available (Van Groenigen et al. 2006). Short-term experiments frequently show a positive effect of elevated atmospheric [CO_2] on symbiotic N_2 fixation, but in the long run, a reduced availability of, in particular, Mo, a key component of nitrogenase, leads to rates of N_2 fixation similar to those under ambient [CO_2] (Hungate et al. 2004) .

3.9 Suppression of the Legume–Rhizobium Symbiosis at Low pH and in the Presence of a Large Supply of Combined Nitrogen

Once rhizobia have successfully infected legume roots and nodules have been formed, further infection is suppressed. This is called **autoregulation** of root nodule formation, a phenomenon we encountered before in the establishment of a mycorrhizal symbiosis (Sect. 2.3.1). Using plants grown with a divided root system, it can be shown that autoregulation depends on **systemic signals** (Van Brussel et al. 2002). Infection of one root of *Vicia sativa* (common vetch) with *Rhizobium leguminosarum* bacteria inhibits nodulation of a spatially separated root, when this root is inoculated 2 days later with the same bacteria. The mechanism by which nodulation is autoregulated is related to that by which combined N inhibits nodulation (see below). Genes that are involved in Nod-factor signaling may be targets for mechanisms that suppress nodulation (Limpens & Bisseling 2003).

At **low pH**, nodule formation on legumes tends to be inhibited. Because fixation of N_2 lowers soil pH (Sect. 3.1 of Chapter 6 on mineral nutrition), continued use of legumes in agriculture requires **regular liming**. Why is nodulation impaired at a low soil pH? The assay system described in Sect. 3.3.1 has been used to establish that a relatively acid or alkaline, as opposed to a neutral pH of the soil, leads to less effective root exudates. This offers an explanation for the common observation of a poor infection of legumes by rhizobium in acid soils. Survival of rhizobia is also lower in soils with a low pH, but some degree of adaptation of rhizobia strains has been observed.

N_2 fixation is an energetically expensive process, as compared with the assimilation of NO_3^- or NH_4^+. Reminiscent of the effect of P_i on the formation of the mycorrhizal symbiosis, **NO_3^-** often inhibits the **infection** of legumes by rhizobia, but this is not invariably found (Sprent 1999). When *Medicago sativa* (alfalfa) plants are grown under N-limiting conditions, the expression of the genes involved in flavonoid biosynthesis and the production of root flavonoids are enhanced. This may account for greater infection by *Rhizobium meliloti* under conditions when N is in short supply, as opposed to suppression of nodulation in the presence of high NO_3^- concentrations (Coronado et al. 1995).

Table 9. Apparent nitrogenase activity and the O_2-limitation coefficient, 2 days after addition of NO_3^- to the root environment of nodulated 21-day-old plants of *Pisum sativum* (pea).*

[NO_3] (mM)	Apparent nitrogenase activity [nmol H_2 g^{-1} (nodule dry mass) s^{-1}]	O_2 limitation coefficient
0	45	0.89
5	38	0.64
10	22	0.45
15	24	0.49

***Source*: Kaiser et al. (1997).**

*** The apparent nitrogenase activity was measured as the rate of H_2 evolution. As explained in Sect. 3.4, nitrogenase activity leads to the production of H_2. There is normally no net evolution of H_2, because rhizobia have a hydrogenase (i.e., an enzyme that uses H_2 as an electron donor). In the present experiment, a rhizobium strain was used that lacks hydrogenase so that the evolution of H_2 could be measured. The O_2 limitation coefficient is calculated as the ratio between total nitrogenase activity (H_2 evolution in the absence of N_2) and potential nitrogenase activity (H_2 evolution in the absence of N_2 at an optimum concentration of O_2).**

NO_3^- also inhibits the process of **fixation** itself (Table 9). Several mechanisms have been proposed to account for this inhibition (Hunt & Layzell 1993):

1. Competition for **carbohydrates** between nitrogenase and nitrate reductase, located in leaves, roots, or nodules
2. Inhibitory effects of **NO_2^-**, the product of nitrate reductase. NO_2^- may inhibit nitrogenase directly, by irreversibly binding to the enzyme, or indirectly, by forming a bond with leghemoglobin, so that it can no longer function in O_2 transport
3. A decrease of the partial pressure of **O_2** in the nodule, due to a decrease in the conductance for gas transport in the pathway between the outside air and the infected cells
4. **Feedback inhibition** of nodule metabolism by nitrogenous compounds that arrive via the phloem

There is evidence for a decrease in the conductance for O_2 transport to the infected cells which leads to a more severe limitation of nitrogenase activity by O_2 (Table 9). It is unlikely, however, that this is the only mechanism that accounts for NO_3^- inhibition of nodule activity. Rather, all four mechanisms probably occur at one stage or another in some species.

Several mutants of a number of legumes have been produced, of which neither infection nor N_2 fixation itself is inhibited by nitrate. These mutants are expected to enhance input of N through the legume–rhizobium symbiosis in agricultural systems.

4. Endosymbionts

Many plants are infected by **fungal endophytes** (family Clavicipitaceae, Ascomycota) that live their entire life cycle within a plant (Bacon & De Battista 1991). The fungi form nonpathogenic and usually intercellular associations in living plant tissue. The endophytes are often transmitted through the plant seed, particularly in grasses and sedges, but seeds may lose their endophytes upon prolonged storage. Infection through germinating spores is an alternative way to enter the macrosymbiont. The association between higher plants and endosymbiotic fungi has been well studied in grasses in which the fungi may produce **alkaloids** in the tissue of their hosts, many of which have a neurotoxic effect, and hence make the infected plants poisonous to domestic mammals and increase their resistance to insect herbivores (Table 10).

In some species, plant growth and seed production can be increased by infection with the endophyte. The symbiotic associations between grasses

Table 10. Antiherbivore effects of fungal endophytes that infect grasses.

Animal	Host genus grass	Fungal endophyte genus	Comments
Mammals			
Cattle, horses	*Festuca*	*Acremonium*	Reduced mass gain, gangrene, spontaneous abortion
Cattle, sheep, deer	*Lolium*	*Acremonium*	Reduced mass gain, tremors, staggers, death
Cattle, goats	*Andropogon*	*Balansia*	Reduced milk production, death
Cattle	*Paspalum*	*Myriogenospora*	Reduced mass gain, tremors gangrene
Insects			
Fall armyworm	*Cenchrus*	*Balansia*	Avoidance, reduced survival, reduced growth, increased development time
	Cyperus	*Balansia*	
	Festuca	*Acremonium*	
	Lolium	*Acremonium*	
	Paspalum	*Myriogenospora*	
	Stipa	*Atkinsonella*	
Aphids	*Festuca*	*Acremonium*	Avoidance
Billbugs	*Lolium*	*Acremonium*	Reduced feeding and oviposition
Crickets	*Lolium*	*Acremonium*	Complete mortality
Cutworms	*Dactylis*	*Epichloe*	Reduced survival and mass gain
Flour beetles	*Lolium*	*Acremonium*	Reduced population growth
Sod webworms	*Lolium*	*Acremonium*	Reduced feeding and oviposition
Stem weevils	*Lolium*	*Acremonium*	Reduced feeding and oviposition

Source: Clay (1988).
Note: The examples are representative but not exhaustive.

and fungal endophytes may be an association in which the fungi derive carbohydrates from their host and defend their host against **herbivory**, thereby defending their own resources. Similar to the effect that mycorrhizal fungi have on interactions among mycorrhizal and nonmycorrhizal plants (Sect. 2.2), fungal endophytes may also influence competitive interactions between plants. For example, grass plants infected with fungal endosymbionts are less nutritious (Clay et al. 1993) and preferred less than the uninfected plants of the same species. The presence of endophytes may also affect competition among grasses in interaction with herbivory (Clay et al. 1993) and suppress the fungal take-all disease in *Triticum aestivum* (wheat) (Dewan & Sivasithamparam 1988).

The presence or absence of fungal endophytes is not a specific trait of a plant species; rather, it depends on environmental conditions in an as-yet-unclear manner. For example, in Western Australian heaths (Ericaceae), the number of fungal associates is considerably smaller on a mesic wetland site when compared with a dryland habitat, even when comparing the endophytes associated with the same plant species (*Lysinema ciliatum*). This appears to reflect the response of different fungal endophytes to water stress (Hutton et al. 1996).

Bacteria may also act as endosymbionts. Some plant-growth-promoting endosymbiotic bacteria have already been discussed in Sect. 3.7: *Gluconacetobacter diazotrophicus*, which fixes N_2 in the tissues of *Saccharum officinarum* (sugarcane). Common endophytic bacteria from healthy tubers of *Solanum tuberosum* (potato) belong to six genera (*Pseudomonas*, *Bacillus*, *Xanthomonas*, *Agrobacterium*, *Actinomyces*, and *Acinetobacter*). As discussed in Sect. 3 of Chapter 9C on effects of microbial pathogens, many bacterial endophytes make the host plant more resistant to pathogen attack (induced resistance) or they enhance growth. There are also endophytic bacteria, however, that are plant-growth-neutral or plant-growth-retarding (Sturz 1995).

5. Plant Life Among Microsymbionts

At one stage in the history of plant ecophysiology it may have seemed most appropriate to discuss the mineral nutrition and performance of plants devoid of their microsymbionts. If we wish to unravel basic principles of plant mineral nutrition (e.g., the nature of a NO_3^- transporter or the function of a micronutrient), then this remains a valid approach. If the aim is to understand plant functioning in a real environment, whether a natural ecosystem or an agricultural field, however, then we cannot ignore the existence and overwhelming importance of the microsymbionts that interact with higher plants in an intricate manner. This is most certainly true for mycorrhizal fungi, which affect both mycorrhizal and nonmycorrhizal species, although in a very different manner.

Plant–microbe interactions do not always receive the attention they deserve. Interactions with N_2-fixing symbionts have been the target of plant physiological research for a long time. Why buy N if you can grow your own? In recent years there has been an enormous development in the understanding of signaling between rhizobia and legumes. Similar signaling processes probably exist between other N_2-fixing microsymbionts and their nonlegume hosts, and major progress has been made on signaling between mycorrhizal fungi and their macrosymbiotic partners.

Endophytes other than the "classic" mycorrhizal fungi and N_2-fixing microorganisms include the fascinating N_2-fixing microorganisms in the apoplast of *Saccharum officinarum* (sugarcane) and toxin-producing endophytes in grasses. We are only just beginning to understand the agronomic and ecological significance of these endophytes. Another question that remains to be answered is how symbiotic microorganisms are allowed entry into the plant when we know that plants have a wide array of defense mechanisms to keep microorganisms at bay.

In this chapter we have showcased one of many areas in plant physiological ecology where "established" terms like **ecology** and **molecular plant physiology** have become obsolete. We can only further our basic understanding of interactions between plants and their microsymbionts if we abolish barriers that hinder the developments in this field. Many applications of a basic understanding of symbiotic associations between plants and microorganisms are to be expected.

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